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CLINICAL TRIALS IN RADIOTHERAPY AND THE MERITS OF HIGH ENERGY PROTONS

by

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The technical developments in radiotherapy have largely been aimed at allowing an adequate dose to be administered optimally (homogeneously or graded) to a volume suspected of tumour growth while avoiding excessive dose to surrounding tissue With the ^{60}Co-sources linear accelerators and betatrons now available at most radiotherapeutic centres, this object is achieved almost to the extent possible with photons and electrons If further advancement along this line of progress is of importance the logical next step would be to exploit ions such as protons accelerated to high energies With this type of radiation the shaping of the radiation field can be made even more sophisticated

High energy protons do not differ appreciably from other low LET radiations with respect to the biological effect and hence do not share the potential capacity of neutrons negative pions and heavy nuclei with respect to the control of anoxic tumours On the other hand the biological effects can probably be modified by temporal and chemical means in the same manner as the effects of other low LET radiations

During the last ten years, 185 MeV protons have been used for radiotherapy on a moderate scale at the Gustaf Werner institute The experience is that the

From the Gustaf Werner Institute University of Uppsala Sweden Submitted for publication 2 January 1969

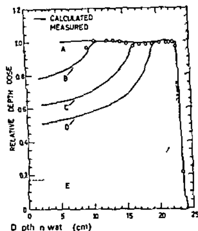


Fig 1 Depth do e curves for 185 MeV protons un modified (E) and modified by the use of ridge filters (A—D) (From KARLSSON 1964 Courtesy Strahlentherapie)

thickness in the beam the peak can be swept in the axial direction over a suitable depth The water absorber can be replaced by ridge filters which introduce different thicknesses of absorbing material in different parts of the beam (LARSSON 1961 KARLSSON 1964) Homogeneous depth dose distributions are obtained by a transversal oscillation of the filter A typical dose distribution of regular shape measured in a water phantom is given in Fig 2

The LET distribution of high energy protons (> 15 MeV) is nominally of the low LET type (< 3 5 eV/nm) (ICRP ICRU 1963) Recoil protons as well as nucleons and nu lei emitted in nuclear reactions add a small high LET com

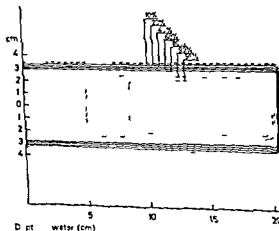


Fig 2 Depth-dose distribution for 185 MeV protons in water after a heavy alloy filter giving a 23 cm plateau

achieved dose distributions are favourable as judged by conventional standards, that the clinical results are comparable to those obtained with other types of radiation, and that no extra risks are involved, neither with respect to untoward effects nor to the safety of patient and personnel (FALKMER et coll 1962, FORS et coll 1964, GRAFFMAN et coll 1965) Contradicting evidence has not been *reported from the other places where therapeutic irradiations by high energy* protons have been performed (KJELLBERG et coll 1962, 1965, 1966, 1967, TOBIAS et coll 1964, 1967)

No strict quantitative study of how high energy protons compare with other types of radiation with respect to clinical results has been made The irradiation facilities and the patient material have not allowed clinical trials (PATERSON 1958) to be set up and no other scientific method appears to be available

It has been our aim to evaluate under what circumstances a clinical trial would be practical To this end the relevant knowledge of the physical, biological and clinical properties of high energy protons was summarized and a number of tentative dose distributions constructed With this approach, no quantitative *estimate could be given, however, of the difference in clinical result that can be* expected from the use of high energy protons as contrasted to other high energy radiations of more common use The problem was therefore attacked by a rather general tumour therapy model and also by re assessment of data from published clinical trials

Physical properties of the high energy proton radiation field The monoenergetic proton beam has a depth dose distribution of the Bragg type (cf Fig 1) The lateral scattering is small and in a first approximation the protons can be considered as moving in straight lines for the full penetration depth The range of secondary particles, except those created in the rather few nuclear reactions, is less than 0 6 mm in soft tissue The contribution to the dose from nuclear reactions is comparatively small both as regards secondary radiation ($\approx$ 20 %) and remaining radioactivity ($\approx$ 0 2 %) (HALLER & JUNG 1963 JUNG 1963, GRAFFMAN & JUNG 1967) The cross section of the field is easily defined by collimators, 50 mm of brass stopping a 185 MeV beam completely The maximum penetration depth in tissue of such a beam is 23 cm

The proton fluence over the utilized cross section can be made homogeneous by scatterers or by sweeping a pencil shaped proton beam in a reticular pattern with two variable, *crossed magnet fields In the latter case a transversal homo*

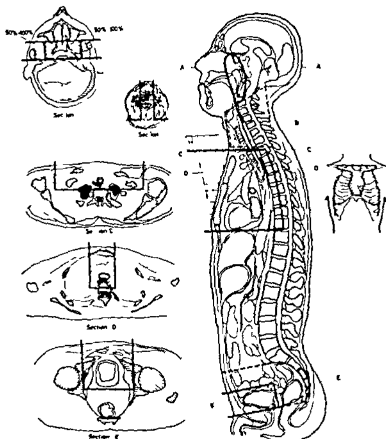


Fig 4 Schematic dose distributions for the treatment of nasopharyngeal oesophageal and bladder tumours

The dose to the lateral parts of the head can be $< 50\%$ of the dose to the nasopharynx if two opposing and overlapping fields with e g a 5 cm plateau are used

The broken line in the neck region indicates the field for irradiating the lateral lymph nodes This can be achieved either by a split frontal field or by two continuous opposing lateral fields covering both the nasopharynx and the neck nodes

The hatched area in front of the thorax symbolizes a mould

The broken line in the pelvic region indicates the extension of the proton field for irradiation of large pelvic tumours

The heavy or broken lines indicate the extension of the homogeneous radiation field carrying the full tumour dose unless otherwise is specified

such as ^{60}Co photons It can also be noted that superficial tissue seems to well tolerate the constant-dose proton field when in the therapeutic dose interval

More conclusive evidence has been obtained in animal and plant experiments It has been demonstrated that the RBE value for high energy protons when compared to ^{60}Co photons, is close to 1 0 for most of the systems studied (cf

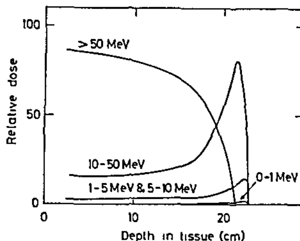


Fig 3 Depth dose curves for various LET components for 185 MeV protons in soft tissue after a heavy alloy filter giving a 23 cm plateau The calculations are based on ZERBY & KINNEY (1965) The curves for 5 to 10 MeV and 1 to 5 MeV protons are identical

ponent In the last few millimetres of their range the protons have themselves a high LET The ridge filters and the variable water absorber modify the proton spectrum so that protons stop everywhere in the plateau region More protons stop in the deeper layers and the high LET proton component hence increases with depth The dose fractions due to protons of various energies is given in Fig 3 as a function of depth in a flat dose distribution The curves are based on data given by ZERBY & KINNEY (1965)

Knowing the appropriate RBE value in the different parts of the Bragg curve it would be possible to correct the shape of the filters so that the resulting depth dose distribution gives a constant biological effect in the plateau region The increase in RBE is probably of importance only in the last few millimetres of the field in which the dose is already decreasing (GRAFFMAN et coll 1967)

In conclusion, high energy protons offer an outstanding flexibility in the shaping of the dose distribution The beam cross section as well as the surface of maximum penetration of the field can easily be formed to an intricate shape by appropriate diaphragms and range determining moulds The depth distribution can be varied continuously from one of the Bragg type to one of constant dose

Radiobiological information on the effect of high energy protons The use of high energy protons in radiotherapy has been limited and hence the information is meagre regarding their radiobiological effects in therapy, a fact which is further accentuated by the difficulties met with in the interpretation of the results From the experience in Uppsala, it may be stated, however, that no clinical effects have been encountered which would indicate a marked dissimilarity in radiobiological effect between high energy protons and other low LET radiations

etration depths at the different parts of the two opposing fields Also tumours within other parts of the pharynx can of course, be irradiated according to similar principles

Oesophagus and pulmones Since more than 40 % of the tumours in the oesophagus have an early lymphatic spread to mediastinal nodes (MERENDINO et coll 1952, TOMLINSON & WILSON 1945) there are often good reasons to include the whole mediastinum in the primary radiation field This can be achieved with a single field as indicated in Fig 4 The dose to the spinal medulla is negligible and only a very small part of the lung is irradiated The integral dose is reduced to about 1/10 of the integral dose in conventional therapy (PIERQUIN et coll 1966, LEDERMAN et coll 1966) although a larger volume is given full tumour dose However a considerable part of the heart is included in the frontal proton field and an opposing back field may be advisable The risks of heart injury have been discussed by e g MICHAILOV (1963) and STEWART et coll (1967) and according to their data the back field should carry about 30 % of the tumour dose The dose to the spinal medulla given by this back field is well within the tolerance limits In the treatment of bronchial carcinoma a similar but adequately extended field arrangement could be used

Vesica urinalis and uterus Bladder tumours can be treated with a single field of protons without considerable irradiation of the rectal mucosa When there is extended growth with regional lymphatic spread the whole pelvis can be irradiated with a broad single field This technique has been applied in the treatment of cervical carcinoma (FALKMER et coll 1962 and FORS et coll 1964) In this case extra bolus might be added to lower the dose to at least part of the rectal region

Quantitative aspects on beneficial effects from proton radiotherapy

The four criteria for field selection seem to be generally accepted but there is a remarkable lack of quantitative reports of their importance in the literature

Disregarding with reference to an earlier section any radiobiological differences between high energy protons electrons and gammas, the major merit of the proton radiation field lies in its geometrical properties These often allow the primary tumour to be given a homogeneous dose with very little dose to most of the surrounding tissue and the regional metastases to be better covered by the radiation field There appears to be no quantitative report in the literature on the importance of these points Of course some kind of cell survival model could be applied but as has been shown by e g SUIT et coll (1967), the result of such model calculations often does not agree with the clinical experience A

e g BONET MAURY et coll 1964, JOHANSSON & LARSSON 1968, LARSSON 1967, STENSON 1969) It thus appears both reasonable and safe to adopt an RBE value of 1 0 ± 0 2 for use in tumour therapy although there are some reports of substantial differences from this value (BONET-MAURY et coll 1964)

The oxygen enhancement ratio for high energy protons is close to what has been found for other low LET radiation (LARSSON & KIHLMAN 1960, LARSSON & STENSON 1965)

Dose distribution obtainable with high energy protons in radiotherapy

A number of tentative dose distributions for various tumour localizations were constructed in order to demonstrate possible applications of high energy protons in radiotherapy The following criteria were applied in the selection of the fields (1) the dose to critical organs must not exceed adopted tolerance levels, (2) the dose to the primary tumour volume should be tumouricidal as judged by current standards, (3) the region susceptible of lymphatic spread should, if so is considered appropriate, receive a tumouricidal dose and (4) the anticipated damage to normal tissue, and to critical organs in particular, should be as small as possible

The resulting dose distributions are shown in Fig 4 The low dose to critical organs, the low integral dose and the outstanding capacity of giving full tumouricidal dose to the primary tumour and to the regional lymphatic system without violating restrictions posed by tolerable integral doses or anticipated damage to critical organs should be noted The applications of proton fields to tumours of superficial localization is not examplified but it is obvious that for e g breast tumours the capacity of a total avoidance of lung and heart irradiation should be of clinical importance

The individual dose distributions are commented on below

Pharynx Because of their deep localization, their closeness to vulnerable organs such as the eyes and the medulla and their extremely high tendency of regional lymphatic spread nasopharyngeal tumours are one of the technically most difficult treatment objects in the head and neck region The problems involved have been discussed in detail by e g LEDERMAN & MOULD (1968) and MILLION et coll (1963) Both groups recommend two opposing fields to the nasopharyngeal region and an anterior split field to the neck nodes Such a dose distribution is easily achieved with high energy protons but a field arrangement as illustrated in Fig 4 ought to have a higher probability of covering all lymph nodes Bolus of rather intricate shape has to be applied in order to achieve the correct pen

Table 1

Probability of regional (p_2) and distant (p_3) spread of various tumour types

Reference	Tumour localization	p	p	Comments
MERENDINO & MARK 1952	Oesophagus	0 40	0 32	Squamous cell carcinoma operation or autopsy material
TOMLINSON & WILSON 1945	Oesophagus	1 00		Squamous cell carcinoma autopsy material
BLOEDORN 1966	Bronchus	0 70	(0 85)	All histologic types operation material p estimated from f equency of blood vessel invasion
HORWITZ et coll 1965	Bronchus		0 80	All patients at admiss on
LARSSON & ROMANUS 1963	Cutis	0 08		
	Labium	0 13		
	Larynx	0 15		
	Lingua	0 45		
	Hypopharynx	0 45		
	Floor of mouth	0 55		
	Tonsilla	0 75		
MILLION et coll 1963	Tons lla	0 74		Unilateral spread p=0 63 bilateral spread p=0 11
	Base of tongue	0 77		Unilateral spread p=0 42 bilateral spread p=0 35
	Nasopharynx	0 90		Unilateral spread p=0 39 bilateral spread p=0 51
LEDERMAN 1961	Nasopharynx	0 70		
LENZ 1942	Nasopharynx	0 53	0 29	
REIFFENSTUHL 1967	Collum uteri	0 19		Stage I
		0 32		Stage II
		0 53		Stage III
		0 67		St ge IV
KOTTMEIER 1964	Collum uter		0 02	Stage I from mate ial with primary heal ng of primary tumour
			0 05	Stage II f om material with primary healing of primary tumour
			0 09	Stage III from material w th primary h al ng of primary tumour
			0 11	Stage IV from material with primary healing of prima y tumour

quantitative study of the frequency of cases where a curative treatment is not given because of anticipated damage to critical organs due to limitations in the shaping of the radiation field would be of special interest in this context, since the major merit of high energy protons may be to reduce this frequency.

The integral dose has often been referred to as some kind of a figure of merit for comparing different techniques of dose administration. To our knowledge, it *has not been possible, however, to demonstrate, on a patient material,* the influence of the integral dose, as distinct from local dose, on the clinical result (cf. e.g. KOHN et coll. 1965). There is a tendency among radiotherapists, however, to prescribe a lowered tumour dose or an extended treatment period when the integral dose is extraordinarily high or when the patient is in a weak condition and hence would not tolerate the effect of, possibly, the integral dose. This would imply that a larger number of the patients could be curatively treated with a radiation type that gives a low integral dose. There appears however to be no way of assessing quantitatively, from literature data, the importance of this fact. If there is a natural defence mechanism against neoplastic cells (cf. e.g. SUIT et coll. 1967) its effectiveness might be correlated to the integral dose. Probably, the correlation is negative although the opposite cannot be excluded (SIKOV et coll. 1964).

Estimate of difference in cure rate between two therapy procedures

As the beneficial effects of high energy protons cannot be estimated directly from empirical findings, a simple tumour model was constructed as an aid in the estimation of the difference in cure rate that can be expected between two *therapy methods of which one may be proton therapy.* Such an estimate is, of course, necessary for the planning of a clinical trial since it determines the number of patients that one should be prepared to include in the trial.

For the sake of simplicity the possible tumour bearing sites in the patient are divided into three volumes, a primary one including the primary tumour in its full extension, a secondary one covering the regional metastases that can be included in the volume (or volumes) irradiated by full tumouricidal dose and a tertiary volume that cannot be curatively treated by radiotherapy. The model is based on the assumption that the following five conditions are necessary and sufficient for a therapeutic procedure to be curative: (1) all parts of the primary tissue that are within the irradiated volume must be controlled, (2) there shall be no primary tumour growth outside this volume, (3) all parts of the regional metastatic spread that are within the irradiated volume must be controlled, (4) there shall be no regional metastases outside this volume, and (5) there shall be no tertiary spread of tumour.

Table 3

Local cure rates of primary tumour ($\alpha_1\beta_1$) and regional metastasis ($\alpha_2\beta_2$) frequency of regional (p_2) and distant (p_3) spread and cure rate (Q) as calculated by the model formula Data taken from Tables 1 and 2 ΔQ_1 and ΔQ_2 are calculated increases in Q if $\alpha_1\beta_1$ and $\alpha_2\beta_2$ respectively are increased by (1 given value)/2

Tumour localization	$\alpha_1\beta_1$	$\alpha_2\beta_2$	p_2	p_3	Q	ΔQ_1	ΔQ_2	Comments
Bronchus	0 35	0 69	0 70	0 85	0 04	0 04	0 01	
Nasopharynx	0 63	0 55	0 80	0 29	0 29	0 08	0 08	
Collum uteri	0 99	0 84	0 19	0 02	0 94	0 00	0 01	Stage I
	0 88	0 56	0 37	0 05	0 77	0 05	0 06	Stage II
	0 57	0 70	0 53	0 09	0 44	0 17	0 04	Stage III

that tumour growth in the primary and secondary volumes situated within the irradiated volume is controlled one finds the cure rate Q to be given by

$$Q = \alpha_1\beta_1(q_2 + p_2\alpha_2\beta_2)q_3$$

where

$$q_2 = 1 - p_2 \text{ and } q_3 = 1 - p_3$$

Admittedly this is a very coarse model but it serves our purpose of demonstrating how a change in *one treatment factor* (e g dose distribution radiation type fractionation regime oxygen tension) may influence the survival of a group of patients The model has the merit of splitting the causes of *treatment failure* into factors depending on the clinical status of the patient the dose distribution and the local treatment efficiency but it neglects the interdependence of the local cure rate and the probability of local and distant spread as well as all negative effects of radiotherapy The formula is easily extended to cover also cases where there are several secondary tumour volumes and of course α and β may as well as p and p_3 be taken to depend on the clinical stage of disease as expressed by e g the TNM classification system Surgical treatment can also be included in the formula

There are few exhaustive reports in the literature on the *patient s clinical status* with respect to actual tumour spread at the time of admittance to the hospital Of course there are good practical reasons for this lack of knowledge For some *tumour localizations* there are however data that may be interpreted to give estimates of the tumour spread A relevant part of this information is given in Table 1

Data on the local cure rate of tumours at the primary site and in the lymphatic

Table 2

Local cure rate for primary tumour ($\alpha_1\beta_1$) and regional metastasis ($\alpha_2\beta_2$) for various tumour localizations

Reference	Tumour localization	$\alpha_1\beta_1$	$\alpha_2\beta_2$	Comments
BLOEDORN 1966	Bronchus	0.35	0.77	At operation after pre-operative radiotherapy
BLOEDORN 1966	Bronchus	0.35	0.60	At operation after pre-operative radiotherapy, regional metastasis verified histologically
SUIT et coll. 1967	Anterior tongue	0.85		Squamous cell carcinoma stage T1—T4, palliative cases excluded
	Floor of mouth	0.90		Squamous cell carcinoma stage T1—T4, palliative cases excluded
	Pharyngeal wall	0.73		Squamous cell carcinoma stage T1—T4, palliative cases excluded
	Hypopharynx	0.70		Squamous cell carcinoma stage T1—T4, palliative cases excluded
	Larynx	0.88		Squamous cell carcinoma stage T1—T4, palliative cases excluded
LENZ 1942	Nasopharynx	0.63		
PERRY et coll. 1966	Metastasis in lymph nodes of neck		0.60	Primary tumour controlled
			0.50	Primary tumour partially controlled
RUTLEDGE 1966	Collum uteri		(0.84)*	Stage I, after radiotherapy, at operation
			(0.56)*	Stage II, after radiotherapy, at operation
			(0.70)*	Stage III, after radiotherapy, at operation
KOTTMEIER 1964	Collum uteri	0.99		Stage I
		0.88		Stage II
		0.57		Stage III

*Figures obtained from data on frequency of patients without regional metastasis after pre-operative radiotherapy and frequency of patients with metastasis at admittance to hospital given in Table 1.

With this assumption and denoting by p_2 the probability that the patient on admission has regional spread and by p_3 that he has distant spread, by α_1 and α_2 the probabilities that all tumour cells in the primary and secondary volumes, respectively, are within the irradiated volume and by β_1 and β_2 the probability

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Local cure rates of primary tumour ($\alpha_1\beta_1$) and regional metastasis ($\alpha_2\beta_2$) frequency of regional (p_2) and distant (p_3) spread and cure rate (Q) as calculated by the model formula Data taken from Tables 1 and 2 1Q₁ and ΔQ₂ are calculated increases in Q if $\alpha_1\beta_1$ and $\alpha_2\beta_2$ respectively are increased by (1 given value)/2

Tumour localization	$\alpha\ \beta$	$\alpha\ \beta$	p	p	Q	ΔQ	ΔQ_2	Comments
Bronchus	0 35	0 69	0 70	0 85	0 04	0 04	0 01	
Nasopharynx	0 63	0 55	0 80	0 29	0 29	0 08	0 08	
Collum uteri	0 99	0 84	0 19	0 07	0 94	0 00	0 01	Stage I
	0 88	0 56	0 32	0 05	0 72	0 05	0 06	Stage II
	0 57	0 70	0 53	0 09	0 44	0 17	0 04	Stage III

that tumour growth in the primary and secondary volumes situated within the irradiated volume is controlled one finds the cure rate, Q to be given by

$$Q=\alpha_1\beta_1(q\ \ + p_2\,\alpha_2\,\beta\ )q_3$$

where

$$q\ = 1 - p_2 \text{ and } q_3 = 1 - p_3$$

Admittedly this is a very coarse model but it serves our purpose of demonstrating how a change in one treatment factor (e g dose distribution radiation type, fractionation regime oxygen tension) may influence the survival of a group of patients The model has the merit of splitting the causes of treatment failure into factors depending on the clinical status of the patient the dose distribution and the local treatment efficiency but it neglects the interdependence of the local cure rate and the probability of local and distant spread as well as all negative effects of radiotherapy The formula is easily extended to cover also cases where there are several secondary tumour volumes and of course α and β may, as well as p and p_3 be taken to depend on the clinical stage of disease as expressed by e g the TNM classification system Surgical treatment can also be included in the formula.

There are few exhaustive reports in the literature on the patient s clinical status with respect to actual tumour spread at the time of admittance to the hospital Of course there are good practical reasons for this lack of knowledge For some tumour localizations there are however data that may be interpreted to give estimates of the tumour spread A relevant part of this information is given in Table 1

Data on the local cure rate of tumours at the primary site and in the lymphatic

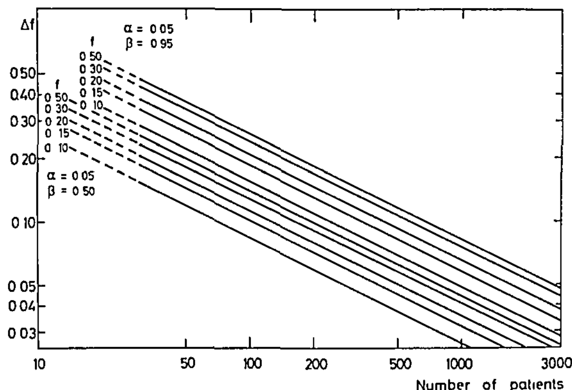


Fig 5 Diagram showing the relation between the expected difference in cure rate (or similar measure) Δf and the number of patients that should be included in each group of a clinical trial in order to have a 95 % ($\beta = 0.95$) and 50 % ($\beta = 0.50$) probability for a significant ($\alpha = 0.05$) result The parameter f is the mean result of the two treatment types

nodes are also hard to obtain from the literature This may partly be due to the fact that *the local cure rate is difficult to define and hence hard to assess* (Similar arguments can, of course, be raised against the whole model, but it is hoped that the lack of stringence is of minor importance in the present context in which only crude estimates are aimed at) From some reported data local cure rates for primary and sometimes also secondary tumours of various location and histologic type can be inferred These are given in Table 2

Also when the information is taken from several sources there are very few tumour localizations for which figures can be given for both the tumour spread and the local cure rate These cases are collected in Table 3, in which also the cure rate obtained by the formula is given, as well as the increase in cure rate that can be expected for half the maximum possible increase in either $a_1\beta_1$ or $a_2\beta_2$, i e an increase by $0.5(1-a_1\beta_1)$ and $0.5(1-a_2\beta_2)$, respectively The high values of Q and ΔQ_1 in Table 3 for stage III of cervical cancer can be explained by the fact that the p_3 value is derived from a material where healing of the primary tumour had occurred

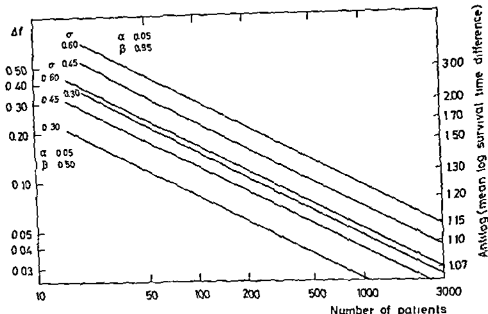


Fig 6 Diagram showing the relation between the expected difference in mean log survival time and the number of patients that should be included in each group of a clinical trial in order to ha e a 95 c ($\beta = 0.95$) and 50 ($\beta = 0.50$) probability of finding a significant ($\alpha = 0.05$) difference in the results The parameter σ is the standard deviation of the log mean survival tim distr bution

No data were found that allow an estimate of α and β separately Both factors are retained in the formula since it is believed that they may be of at least theoretical interest

The data indicate that an increase in Q by 0 10 is a very successful result and that often a much smaller increase must be expected It may well be argued that this is a meagre result of the work spent on the model calculations These are nonetheless retained here since the model at least to us seems to indicate a route by which when the proper basic knowledge has been accumulated it may be possible to estimate with some accuracy the merits of a change in treatment procedure

Statistical procedures

Using the non central F distribution given by e g PEARSON & HARTLEY 1951) it is easy to find with some obvious simplifying assumptions, the relation between the expected difference in survival rate and the necessary number of

Table 4

Results of clinical trials p = reported level of significance f = reported results for the two treatments and Δf = difference that would have been announced as significant (p = 0 05) with a probability of 0 50 and 0 95

Tumour localization and treatment type treatment common to both groups is given before treatment different is separated by /	Measure of result	Mean number of patients in the *two groups*
Bronchial carcinoma		
Surgery/8 MV roentgen	4 years	29
		19
Radiotherapy/surgery	Mean	72
Roentgen 8 MV 3 000 rad/4 000 rad	Mean	51
	2 years	
Pneumonectomy without/with roentgen	3 years	101
Radiotherapy split course/routine	1 year	15
^{60}Co gamma air/3 atm O_2	13 months	25
^{60}Co gamma chlorambucil/placebo	Median	12
Roentgen synkavit + O_2/synkavit	*Mean log*	*68*
/compound 28		57
/O_2		56
Roentgen synkavit + O_2/synkavit		45
/compound 28		37
/O_2		38
Roentgen synkavit/no sensitizer	Mean	42
Roentgen + synkavit intravenous/intramuscular	Mean	28
Roentgen 5 FU/no sensitizer	Mean	13
Roentgen split course cytoxan/5 FU	Mean	7
Buccal carcinoma		
^{60}Co gamma synkavit/no sensitizer	Local healing	4[illegible]
Lingual carcinoma		
^{60}Co gamma synkavit/no sensitizer	Local healing	18
Tumour metastasis in neck region		
Neck dissection 18 MeV eV pre operatively/no radiotherapy	Local healing	61
Bladder carcinoma		
^{60}Co gamma air/3 atm O_2	13 months	20
Cancer mammae		
Surgery with/without immediate radiotherapy	5 years	355
		370
Extended radical mastectomy with/without roentgen	5 years	153
		89
Cancer cervix uteri		
Radium after/before roentgen	5 years	24
		87

Table 4 *(cont)*

P	C	Δf		Comments	Reference
		0 50	0 95		
—	0 23/0 07	0 19	0 35	Operable carcinoma all histologic types	1
0 05	0 30/0 06			Operable squamous cell carcinoma	
0 05	256d/190d			Oat celled carcinoma	2
0 05	9 3m 6 0m			Inoperable anaplastic carc noma	3
—	0 06/0 00				
—	0 36/0 33	0 13	0 25		4
—	0 20 0 07	0 3	0 5	Inoperable squamous cell carcinoma	5
—	0 25/0 20	0 23	0 46		6
0 001	7 4m/5 0m				7
—	0 69/0 67	0 13	0 2?	Inoperable tumours males	8
—	/0 65	0 13	0 24		
—	/0 63	0 13	0 24		
—	0 73/0 66	0 14	0 27	Histological verified tumours	
—	/0 68	0 15	0 29		
0 05	/0 5?	0 15	0 29		
0 001	8 7m/3 8m				9
0 01	11m 5 9m				
0 05	17m/6 2m			Inoperable carcinoma	10
—	6 8m/5 8m				
0 1	0 41/0 ?0	0 19	0 37	Resolution and no recurrence	11
–	0 23 0 09	0 2	0 4	Resolution and no recurrence	
0 05	0 13 0 33	0 15	0 29	No recurrence within 1 year	1?
	0 33 0 1?	0 3	0 5		6
	0 45 0 44	0 07	0 14	Irradiat on of operat on flap and axilla	13
	0 43 0 39	0 07	0 13	Irrad at on of regional lymph nodes	
	0 64 0 [illegible]	0 11	0 21	All ope able cases	14
–	0 [illegible] 0 71	0 13	0 25	Clinical stage I	
	0 33 0 44	0 3	0 5	Clinical stage II	15
0 07	0 48 0 33	0 15	0 ?7	Cl cal stage III	

Table 4 *(cont)*

Tumour localization and treatment type treatment common to both groups is given before , treatment different is separated by/	Measure of results	Mean number of patients in the two groups
Radium with/without roentgen	5 years	187
Radium and external irradiation kilovoltage/megavoltage	3 years	147
Radiotherapy with/without lymphadenectomy	5 years	35
		39
		27
		26
		30
^{60}Co gamma conventional/grid technique	1 year	15
Cancer of pharynx larynx and bladder		
Roentgen progressive dose/even dose	5 years	154
Cancer of upper digestive and respiratory passage		
Roentgen 4 MV 4 atm O_2/air	6 months	15

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1 MORRISON et coll 1963
2 DEELEY 1967
3 DEELEY 1966
4 PATERSON & RUSSEL 1962
5 LEVITT et coll 1967
6 CADE & MCEWEN 1967
7 HORWITZ et coll 1965
8 MITCHELL & HAYBITTLE 1965
9 MITCHELL 1953
10 GOLLIN et coll 1964

patients in each group of a clinical trial for a 95 % or 50 % probability of a result that is significant on the 5 % level (cf Fig 5)

When the aim of a radiotherapeutic procedure is palliation, the survival time might be a relevant measure BOAG (1948, 1960) and others (e g KAUFMAN 1966, LENARDUZZI et coll 1967) have demonstrated that for fatal patients the logarithms of the survival times are approximately normally distributed The standard deviation of the distribution is about 0 45 when logarithms to the base of 10 are used In Fig 6 are given curves which correlate the necessary number of patients for 95 % and 50 % probability of a significant result and the expected difference in mean log survival time The curves are based on the non central F distribution

Re assessment of clinical trials

In a large number of the fully analyzed clinical trials the verdict has read 'difference not significant Since this statement tells little about the magnitude

Table 4 (*cont*)

p	f	Δf 0 50	Δf 0 95	Comments	Reference
—	0 62/0 59	0 10	0 19	Clinical stage I and II	16
—	0 68/0 68	0 10	0 20	Clinical stage I and II	
—	0 93/0 86	0 14	0 26	Clinical stage I	17
—	0 89/0 77	0 14	0 25	Clinical stage IIa	
—	0 62/0 54	0 25	0 50	Clinical stage IIb	
—	0 33/0 54	0 24	0 50	Clinical stage IIIa	
—	0 63/0 54	0 25	0 50	Clinical stage IIIb	
0 007	0 69 0 07			Clinical stage III and IV	18
—	0 35/0 33	0 10	0 20		19
—	0 80 0 64			4×725 rad/4×775 rad for O_2/air	20

11 SHANTA & KRISHNAMURTHI 1964
12 HENSCHKE et coll 1964
13 PATERSON & RUSSEL 1959
14 KAAE & JOHANSEN 1962
15 PATERSON & RUSSEL 1962
16 PATERSON & RUSSEL 1964
17 RUTLEDGE et coll 1965
18 DEED & BARNHARD 1964
19 PATERSON & RUSSEL 1962
20 VAN DEN BRENK 1968

of any real difference that could have remained undetected or that should have been detected with a fair probability we have collected in Table 4 the clinical trials in radiotherapy that we could obtain reports on From the number of patients entering the trial the difference (Δf) that should have given a significant difference (on the 5 % level) in 50 % and 95 % of the trials can be read from Figs 5 and 6 for those trials in which the measure was survival rate or mean log survival time Most of the authors split the patient material in various subgroups according to age clinical status and histologic type of tumour and hence it has not been possible to include all reported results in the table We have tried to limit the presentation to those data which we believe illustrate best the outcome of the trial

It was found that in most of the trials differences as large as 0 20 in survival rate could not be excluded (the criterion for exclusion being a probability of less than 5 % for a non significant difference in results) and in no case could a 50 % chance for detection of a 0 05 difference be attained

There is only one case (MORRISON et coll 1963) in which a significant

difference in cure rate after a reasonable length of time has been announced. The reported difference in 4 year survival is 0.14. In two other cases (MORRISON et coll., PATERSON & RUSSEL 1962) an almost significant difference has been announced. The differences in survival rate were 0.16 and 0.15, respectively. It was concluded that the estimate of difference in cure rate between two therapy protocols arrived at, (see p. 13) is valid, remembering the fact that the habit of contrasting the patient material in several different ways increases the risk for errors of the second type, and taking into account also the differences found in not significant trials. In fact, it can be demonstrated with a χ test that the cohort of clinical trials is not at variance with the null hypothesis that in no trial was there any difference between the two regimes.

There is only one trial in which the measure was the mean log survival time. A significant difference was announced for one of the contrasted pairs of protocols. The difference in mean log survival time was in this case 0.21. It is meaningless to draw any conclusion from this single case as to what difference in mean log survival time could be expected in other trials.

The information from the remaining clinical trials collected in Table 4 does not lend itself to the type of analysis attempted here.

Conclusions

With a blunt formulation it may be stated that future technical advancements and changes in techniques are not likely to bring about, in one single step, a substantial improvement in the clinical result of radiotherapy. The complexity of tumour therapy, the access to modern apparatus and the body of information, acquired through clinical experience and substantiated by follow up studies act in concert to this end. Rigid statistical methods are thus necessary if the small gains in survival rate that can be hoped for shall be detected. Excluding factorial designs as being much too unwieldy, though perhaps more efficient, only clinical trials remain.

It seems to be generally agreed that clinical trials are ethically allowed only when the investigator does not know beforehand which of the two protocols gives the better result. Follow up studies from various radiotherapy clinics indicate that for well classified patient materials the treatment results are remarkably similar irrespective of environmental factors and the admittedly marginal differences in treatment techniques. This would imply that the uncertainty in the estimate of clinical result of a conventional protocol is low, say below 0.10 in survival rate. The uncertainty in the estimate for the contrasted protocol should be of the same magnitude. This would imply that the very method of clinical trial, at the present stage of development, restricts the likely difference in clinical

result to values similar to those indicated by the calculations with the tumour therapy model and by the outcome of the analysis of published clinical trials

Taking into account the complex interplay between all the factors controlled by the therapist essential improvements should still be achievable Each step necessitates however a number of patients often of the order of 1 000 or more Only at very large therapeutic centers or through inter clinical cooperation meticulously planned and carried out can these numbers be collected within a reasonable length of time

As to high energy protons applied on a moderate scale it seems obvious that for patients that can be given full tumour dose with modern conventional equipment the possible merits will be very hard or indeed impossible to assess There may remain however a sizable class of patients to which a homogeneous full tumour dose can be given only with protons and not with common types of radiation due to the geometrical differences of the radiation fields Were a trial confined to such a restricted group of patients the difference in clinical result might be quite large and it may in fact be questioned whether such a trial would be ethical The literature gives, however very little guidance in these matters as the palliatively treated patients often are excluded in the follow up studies or referred to one single group

The discussion of clinical result has been confined mainly to the cure rate this measure being the best available today or at least the one most wideley accepted It does not however take the untoward effects of radiotherapy into proper account but probably better so than the other two measures discussed namely the local cure rate and the mean log survival time The latter measure furthermore is less well suited when there is a substantial cure rate

If other valid and more sensitive measures of both negative and positive effects were available the rate of progress might be considerably speeded up

SUMMARY

The physical radiobiologic and radiotherapeutic properties of high energy protons are summarized and tentative dose distributions for the treatment of tumours in the pharynx oesophagus lungs, urinary bladder and uterus constructed The dose distributions conform well with established criteria but the beneficial effects to be gained from them cannot be assessed quantitatively A crude tumour model indicates that a 0 10 difference in cure rate between two therapy protocols is more than can be expected in most cases Reassessment of results from clinical trials seems to confirm this estimate Other measures of the clinical efficiency such as the mean log survival time and the local response may be more sensitive but are probably not good measures of the effect searched for The conclusion is that of the order of 1 000 patients should be entered in each group of a clinical trial if the difference that can be expected is to be demonstrated significantly with a fair probability

difference in cure rate after a reasonable length of time has been announced The reported difference in 4 year survival is 0 14 In two other cases (MORRISON et coll, PATERSON & RUSSEL 1962) an almost significant difference has been announced The differences in survival rate were 0 16 and 0 15, respectively It was concluded that the *estimate of difference in cure rate between two therapy* protocols arrived at, (see p 13) is valid, remembering the fact that the habit of contrasting the patient material in several different ways increases the risk for errors of the second type, and taking into account also the differences found in not significant trials In fact, it can be demonstrated with a χ^2 test that the cohort of clinical trials is not at variance with the null hypothesis that in no trial was there any difference between the two regimes

There is only one trial in which the measure was the mean log survival time A significant difference was announced for one of the contrasted pairs of protocols The difference in mean log survival time was in this case 0 21 It is meaningless to draw any conclusion from this single case as to what difference in mean log survival time could be expected in other trials

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ZUSAMMENFASSUNG

Die physikalischen radiobiologischen und radiotherapeutischen Eigenschaften hochenergetischer Protonen werden zusammengefasst und versuchsweise Dosisverteilungen fur die Behandlung von Tumoren des Pharynx des Oesophagus der Lungen der Blase und des Uterus konstruiert Die Dosisverteilungen stimmen gut mit den aufgestellten Kriterien uberein aber die vorteilhaften Effekte die daraus gewonnen werden konnen nicht quantitativ eingeschatzt werden Ein grobes Tumormodell deutet darauf hin dass eine 0 10 Differenz der Heilungsrate zwischen zwei Therapieprotokollen grosser ist als sie in den meisten Fallen erwartet werden kann Die Wertung der Resultate klinischer Versuche scheint diese Schatzung zu bestatigen Andere Masse der klinischen Wirkung wie die mittlere logarithmische Überlebenszeit und die lokale Reaktion mogen empfindlicher sein sind aber wahrscheinlich keine guten Masse des gesuchten Effekts Daraus folgert dass jede klinische Versuchsgruppe die Grossenordnung von tausend Patienten umfassen sollte wenn man die Signifikanz der zu erwartenden Differenz mit annehmbarer Wahrscheinlichkeit nachweisen will

RÉSUMÉ

Les auteurs resument les proprietes physiques radio biologiques et radiothérapiques des protons de haute énergie et construisent à titre d essai les distributions de dose pour le traitement des tumeurs du pharynx de l oesophage des poumons de la vessie et de l utérus La distribution de dose concorde bien avec les criteres établis mais l amelioration qui en resulte ne peut etre jugee quantitativement Un modele rudimentaire de tumeur montre qu une différence de 0 10 dans la dose de guérison entre deux protocoles de traitement est plus qu on ne peut attendre de la plupart des cas L etude des resultats d essais cliniques parait confirmer cette estimation D autres mesures de l efficacité clinique telle que le logarithme de la moyenne de la durée de survie et la réponse locale peuvent etre plus sensibles mais ne sont probablement pas de bonnes mesures de l effet étudié La conclusion de cette etude est que chaque groupe d essai clinique devrait comprendre environ 1 000 malades pour qu on puisse mettre en évidence avec une bonne probabilité la difference prévue

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Table

Percentage output for various field areas normalized at the largest measured field area

Area in cm	4×4	5×5	7×7	10×10	15×15	20×20	25×25
Unit A	91 5	92 5	94 0	96 0	98 5	99 0	100
Unit B	74 0	83 0	88 5	93 5	97 5	99 0	100

metry The accuracy of standard and sub-standard measuring equipment, coupled with the knowledge of essential factors determining the dose of radiation permits the calculation of physical data in radiation dosimetry to probably better than ± 3 % There is no doubt that this accuracy is maintained in good radiotherapy centers where in addition constant effort is being made to reduce to a minimum the phantom patient differences and thus provide realistic patients dose distribution However it seems to us that many cobalt users tend to over simplify or disregard various physical components and by doing so might easily commit errors approaching or even exceeding in value those associated with inherent clinical and biologic estimates

It is a matter of common knowledge that the dosimeters for the measurement of high energy radiation are calibrated in equilibrium conditions by the National Bureau of Standards in U S A It is also well known that the delivery of a cobalt 60 source is accompanied by manufacturer s certificate stating the strength of the source in rhm However it is often over looked that the National Bureau of Standards calibrates the Victoreen ionizing chambers in a large radiation field of 500—600 cm and the manufacturers data equally refer to the maximum field size of 25 cm × 25 cm Somehow it is still traditionally believed that the fundamental calibration data refer to the area of 10 × 10 cm this simple misunderstanding might result in dose inaccuracy up to 7 % on some cobalt units (see Table) In computing doses delivered to patients in cobalt teletherapy surface backscatter factors are often disregarded It is true that these factors are much lower than the ones used in conventional deep therapy However a look in the depth dose charts for cobalt 60 will show that the backscatter factor reaches the value 4 6 % for a field size of 20 cm × 20 cm Large fields are used quite often and form an important part of our non conventional techniques there is no reason to neglect a well established and calculated correction factor in cobalt dosimetry

The distribution of the radiation beam across the irradiated field cannot be anticipated but has to be measured in conditions of actual irradiation patterns Furthermore these measurements should be carried out through different depths

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DOSIMETRY OF IRREGULAR FIELDS IN COBALT 60 THERAPY

by

I. M. Aral, D. Cah, M. Nissel and J. Spira

In clinical work involving the use of ionizing radiation, the application of conventional or non conventional (irregular fields) in supervoltage therapy must be accompanied by an adequate knowledge of physical parameters. Unfortunately, considerable differences often exist between various centers in the methods of estimating the radiation dose and its distribution. Any attempt to compare results using identical treatment techniques is bound to fail unless it provides enough physical information to allow the reproducibility of the results and their evaluation. This is of paramount importance in the use of the so-called non conventional or irregular fields for which exact physical data form an important criterion of the new medical concepts.

The concept of absorbed dose and the almost universal use of rad in the exchange of medical information, contribute considerably to the understanding of differences in the biologic effects of radiation. However, a rad is as accurate as the measured roentgen; much smaller errors are committed by averaging in supervoltage therapy the rad roentgen conversion factor between bone, fat and muscle tissues than by neglecting certain parameters in roentgen dosi-

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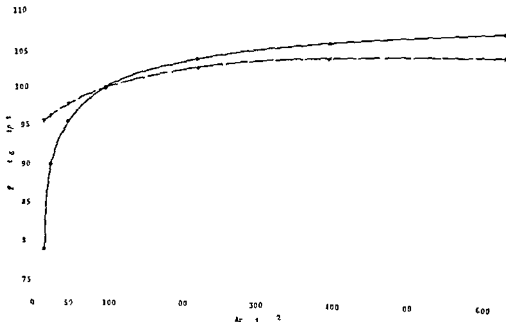


Fig 2 Output of radiation versus field area normalized at 100 cm using cobalt 60 units A (— — — — —) and B (————————)

Twelve Sievert chambers provided with additional lucite caps for electronic equilibrium were exposed in series of multiple consecutive exposures to a calibrated cobalt 60 beam and the sensitivity of each chamber in volts/roentgen was experimentally determined The cobalt 60 doses were measured with the 25 R Victoreen condenser chamber provided with 4 mm lucite cap calibrated by National Bureau of Standards at the field size of 400—500 cm and exposed to the same fields at such a distance as to permit readings of approximately 10—12 roentgens representing the optimum linearity of the 25 R Victoreen chamber The accuracy of the National Bureau of Standards standardization was ± 2 % and the reproducibility of the 25 R chamber was better than ± 1 %

The value of Adlux film in relative dosimetry of supervoltage radiation is well established and the techniques of film dosimetry in these regions have been discussed frequently in scientific publications Finally in order to provide the most realistic approach to dose patient relationship in application of diversified fields of radiation an Alderson phantom was used consistently throughout our investigation This phantom which has been fully described in professional literature allows measurement of radiation doses without errors resulting from the

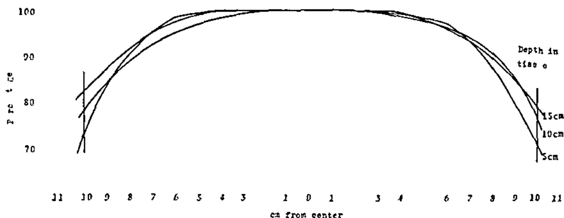


Fig 1 Distribution of dose across the field at different depths with surface field area of 20 cm × 20 cm and using a cobalt 60 unit

in tissue and particularly through the central plane of the tumor It can be shown that differences between the central dose and the dose at the edge of the irradiated field is a function of the depth of tissue the across field becomes more *uniform within the increased depth in tissue (Fig 1) This brings to light another* parameter which should not be neglected in the central dose calculations, frequently, rectangular fields are used with 2 1 or 2 5 1 or even 4 1 ratio of the side lengths The use of equivalent square factors calculated by DAY for the determination of actual depth doses will eliminate a systematic error in patient dosimetry Finally, the relationship between the output in air of cobalt 60 radiation under electron equilibrium conditions and the size of irradiated fields has to be experimentally established We are fortunate to have in our institution two cobalt units which are similar in physical dimensions, source collimator *distance*, size and strength of the source, they *differ considerably only in the* construction of the collimating system Measurements of radiation output in air versus the size of the irradiated field were performed on both units and the enclosed graph (Fig 2) shows the very significant differences It is clear that the change in radiation output with the field area is a function of a number of primary cobalt 60 photons scattered from the walls of the collimator system The significant difference between the two units depends most probably on the type of the collimator and on source collimator geometry We feel that accurate measurements of exposure field area factors should be performed on each cobalt 60 teletherapy unit and that these factors should be used in cobalt 60 dosimetry

All the physical measurements pertaining to this investigation were performed using Sievert miniature ionization chambers in conjunction with the Baldwin Farmer electrometer, Adlux film was used for relative photographic dosimetry

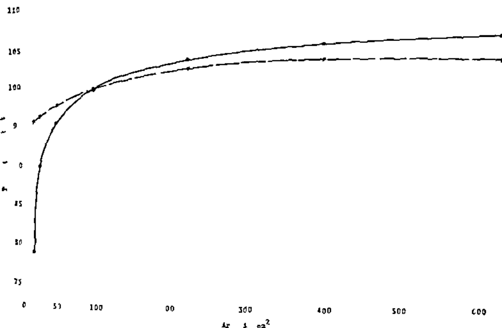


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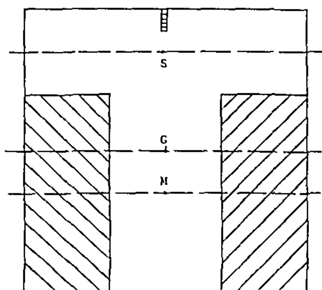


Fig 3 Dose distribution in a T field

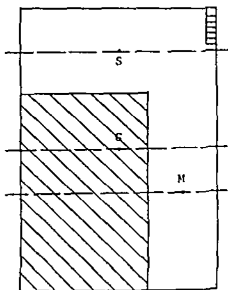


Fig 4 Dose distribution in an L field

varying obliquity of the incident surface and from different densities of the tissues in question

Irregular portals have become quite commonplace in today's techniques for radical radiotherapeutic attacks on disease These bizarre field shaping techniques are demanded because of the difficulty of accurately applying adjacent fields without running into overlap difficulties, or too widely separating fields, with accompanying problems of overdosage and underdosage

We are accustomed in daily use to the application of the following unusual fields a T shaped field in the treatment of malignancies of the mediastinum and supraclavicular regions, an L shaped field for unilateral use in similar clinical situations and in the abdomen, a mantle field for Hodgkin's disease as described by KAPLAN and a diamond shaped field for pelvic irradiation These are obtained by field shaping with lead blocks from rectangular primary areas

Results

The T field (*Fig 3*) In evaluation of the dose reaching the center of the mediastinal field at point *M* from the depth dose charts one must realize that the a p thickness of the patient at the center of the mediastinal field *M*, might be significantly greater than at the geometrical center point *G* of the 25 cm × × 25 cm irradiated field This difference amounted to 5 cm in our phantom set up and the resulting measured dose was 10 % lower than the central depth

dose charts indicated for the position of point *G* To correct this discrepancy one would measure the thickness of the patient through the two points *G* and *M* under identical respiratory condition and use the proper depth for *M* if significantly different

In the center of the upper part of the T field at point *S* at 3 5 cm depth in tissue the radiation dose was found to be 6 % less than the dose obtained from the standard depth dose charts for the particular depth and field area It is significant to note that the dose at the irradiated depth of 3 5 cm in this part of the field drops continuously across the field At 1 cm distance from the edge of the upper rectangle the dose was found to be 70 % of the central dose indicated in the depth dose charts for this area and depth the drop of the dose across the mentioned area is symmetrical

The L field (Fig 4) Results of our measurements show that the dose at point *S* representing the center at 3 5 cm depth of the supraclavicular part of the total irradiated field area of 17 cm × 25 cm is 8 % less than the dose charts for the specified conditions

The dose at the center of the mediastinal field point *M* was found to be 6 % less than the one calculated from the standard depth dose charts for unblocked total area of 17 cm × 25 cm It is interesting to note that identical measurements performed on our second cobalt unit B showed opposite results the dose to point *M* was found to be 5 % higher than the calculated from the depth dose charts The reason for this discrepancy has been experimentally established the distribution across the irradiated area in unit B shows a dip in the center of the field At the present time we have no explanation why in this unit the dose in the center of a field is a few per cent lower than at the points some centimeters around the center The mere fact however proves our previous contention that the physical parameters of each cobalt 60 unit cannot be taken for granted but have to be experimentally established

Mantle field (Fig 5) This field illustrates clearly the possibility of serious clinical errors resulting from the lack of actual dosimetric measurements in an elected treatment pattern Due to the specific geometry of the field the dose at the center of the mediastinal field at point *M* is equal to the dose calculated from the depth dose charts at the center of the 25 cm × 25 cm diamond shaped field at point *G* Furthermore the dose at the center of the supraclavicular field point *S* at 3 5 cm depth is equal to the central depth dose calculated at the same depth for the 25 cm × 25 cm field size However the dose change across the horizontal limb of the mantle is considerable At the clinically important points *A* and *A'* of the axillary region the dose was found to be only 70 % of the calculated dose

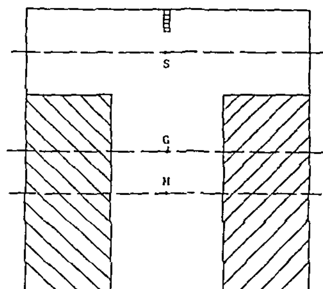


Fig 3 Dose distribution in a T field

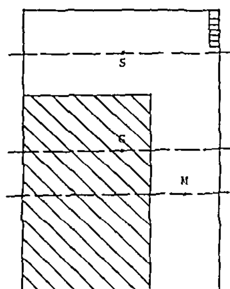


Fig 4 Dose distribution in an L field

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boost up the dose to point B If 4 cm blocks are used the gain at point A is almost 50 % It is certainly of interest to know if in the evaluation of cures following the combined radium and external cobalt therapy this important figure does not really represent the optimal dosage rather than overdosage For all the blocks used in this treatment pattern the dose to point B is 85 to 90 % of the dose delivered at the center of the unblocked field

Conclusion

The concept of dosimetry in application of conventional or irregular fields in cobalt 60 therapy involves rigorous knowledge of physical parameters in determination of the radiation dose delivered to the patient Some of the factors, i e the NBS calibration factor backscatter and the area equivalence corrections have to be properly incorporated in the calculations in others the output versus field area dependence and the dose determination across the irradiated area must be experimentally determined for each cobalt unit In each treatment pattern the distribution of radiation throughout regions of anatomic importance was measured and discussed

From the analysis of the results obtained it is apparent that if published depth dose tables are the sole source of dosage calculation clinically serious regions of over or under dosage may result From our measured data it is manifest that the dosage across large shaped fields and dosage under commonly used blocks cannot be predicted from the tables and should be determined individually for such fields and their respective blocks Once incorporated into a library of dosimetry plans they may be freely applied wherever suitable

SUMMARY

Output versus field size and the dose distribution with cobalt 60 units was experimentally measured for irregular fields commonly used in clinical radiotherapy The results indicate that the dosage across large shaped fields and under commonly used blocks cannot be predicted from tables but should be determined individually for such fields and their respective blocks

ZUSAMMENFASSUNG

Die Ausbeute im Verhaltnis zur Feldgrosse und der Dosisverteilung mit Cobalt 60 Einheiten wurde experimentell fur unregelmassige Felder wie sie gewohnlich in der klinischen Radiotherapie verwendet werden, gemessen Die Ergebnisse deuten darauf hin dass die Dosierung uber gross-formige Felder und bei den gewohnlich verwendeten Blocken nicht aus Tabellen vorherbestimmt werden kann sondern dass diese individuell fur derartige Felder und ihre entsprechenden Blocke bestimmt werden sollte

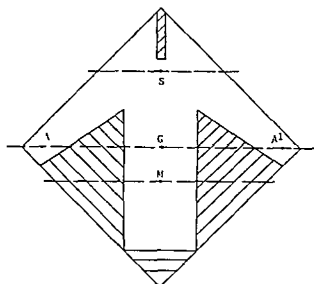


Fig 5 Dose distribution in a mantle field

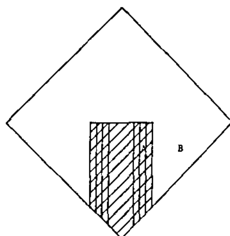


Fig 6 Dose distribution in a diamond shaped field

to the tumor Therefore, we found it necessary to construct special compensators to spread the dose uniformly across the horizontal limb of the mantle field

'Pelvic diamond' field (Fig 6) The diamond shaped field is a square field frequently 13 cm × 13 cm, turned 45° on its central axis perpendicular to the field surface The irradiation field covers the obturator node, (the pelvic walls) the proximal one third of the vagina and the iliac nodes, while leaving the femoral heads outside the irradiated field The diamond shaped field blocked along its midline to prevent over irradiation of the bladder and rectum, is widely applied following the insertion of radium system in the treatment of carcinoma of cervix and uterus The data tabulated below represent the doses received at point *A* while using lead blocks of 5 HVL thickness and different widths of 2 cm, 3 cm, 4 cm and 5 cm measured on the skin of the patient The measured doses are in percentage values of the dose received at the geometrical center of the unblocked field

Width of block	Point A	Point B
2 cm	90	
3 cm	75	85—90
4 cm	45	
5 cm	25	

It will be noted that even with the maximum 5 cm width of the blocks used routinely, point *A* still receives around 25 % of the dose intended primarily to

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DOSE DISTRIBUTION FROM INTRACAVITARY RADIUM AND SUPPLEMENTARY EXTERNAL IRRADIATION WITH REGARD TO TOPOGRAPHY OF LYMPH NODES IN CARCINOMA OF THE UTERINE CERVIX

by

INGEMAR JOELSSON, ANDERS BACKSTROM JAN DIEHL and CURT LAGERGREN

Analysis of the results of radiation treatment of carcinoma of the cervix uteri at Radiumhemmet reveals that during the ten year period 1948—1957 the five year apparent recovery rate was considerably better than during the preceding decade This applied particularly to clinical stages I and II a but less so to the stage II b and III It appears that the improvement may be due at least in part to changes in intracavitary treatment policy with increased regard to the requirements of the individual patient The principles of supplementary external irradiation to the parametrium remained the same

Intracavitary treatment has not been altered since 1957 but the external therapy has been modified significantly For purpose of comparison certain years have been taken as representative For example during the period 1958—19[illegible] conventional roentgen rays were given to the parametrium through four portals

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RÉSUMÉ

Les auteurs ont fait des mesures expérimentales du débit en fonction des dimensions du champ et ont etudie la distribution des doses avec leur appareil de cobalt therapie pour des champs irréguliers utilisés frequemment en radiothérapie clinique Leurs resultats montrent que la dose fournie par de grands champs et avec les blocs utilises habituellement ne peut etre determinee d apres les tables et devrait etre determinee individuellement pour chaque champ et chaque bloc

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ance from serial biopsies during treatment time (KOTTMEIER 1959) The sensitization and radiation responses in carcinoma of the cervix have been thoroughly investigated by KJELLGREN (1958) and RUBIO et coll (1965) All these studies have contributed greatly towards our present understanding of the subject even though the situation is still not clear

The present authors have consequently resorted to the physical aspects and have investigated the dose distribution in the pelvis from routine intracavitary radium treatment Special attention has been paid to the lymph nodes along the iliac vessels which are recognized as highly likely to be involved by carcinoma even in the early clinical stages The topography of these nodes has also been correlated to the tissue volume irradiated homogeneously by supplementary external treatment

Methods Ten patients 5 with stage I b carcinoma and 5 with stage II a were chosen four of the patients had an exophytic tumor growth three had disc shaped tumors and in three patients the carcinoma was endocervical

Lymphography was performed after insertion of a cannula into a lymphatic vessel on the dorsal surface of the foot (RUTTIMANN 1962 TJERNBERG 1962) Lipiodol Ultra Fluide (0 48 mg iodine/ml) was injected at a rate of 0 12 to 0 15 ml/min in a total amount of 8 ml to each side The lymph vessels running along the external and common iliac vessels may be observed during the injection procedures on films taken at this time The location and the characteristics of the lymph nodes may be studied twenty four hours later The external iliac lymph nodes and the common iliac lymph nodes may be subdivided into lateral intermediate and medial groups as already proposed The internal iliac lymph nodes are seldom demonstrated by this technique The subdivisions of the parietal nodes of the internal iliac lymph node system are the superior and inferior gluteal the obturator and the lateral sacral lymph nodes The visceral nodes of the same system are those of the bladder the para uterine tissues and the rectum (KUBIK et coll 1967) In the literature the lymph nodes between the external and the internal iliac arteries are sometimes called the interiliac nodes and are subdivided into the hypogastric nodes in the angle between the external and the internal iliac arteries and the obturator nodes in the obturator fossa cranial to the obturator artery (REIFENSTUHL 1967)

Phlebography was carried out after inserting polythene catheters percutaneously into both common iliac veins One hundred milliliters Urografin 45 % were injected into both tubes at a rate of 5 to 8 ml/sec when the tips of the catheters lay close to the internal iliac veins The inferior vena cava was blocked with a rubber balloon pressed against the abdomen with a plastic plate

Table 1

Corrected survival rate figures for those stage II b and III cases from the consecutive series of carcinoma of the uterine cervix, which were treated with conventional roentgen rays and ^{60}Co two opposed beams and ^{60}Co three beam technique respectively. Frequency of complications from bladder and rectum are given as 3- and 5-year corrected rates for the combined stage II b and III cases using the grading system defined by KOTTMEIER *(1964). Figures obtained from an analysis of case reports at Radiumhemmet* (JOELSSON *1970)*

	Conventional roentgen rays four portals 1958—1959		Cobalt 60 two opposed beams 1960—1962		Cobalt 60 three beam technique 1964—June 1966	
	Stage II b	Stage III	Stage II b	Stage III	Stage II b	Stage III
Number of patients	138	57	42	65	37	71
Percentage of total	72%	42%	16%	42%	22%	58%
3 year corrected survival rate	61%	34%	53%	25%	52%	43%
5 year corrected survival rate	48%	31%	42%	24	—	—
Bladder complications grade II to III						
3 year corrected rate	6%		15%		2	
5 year corrected rate	8%		15%		—	
Rectal complications grade II to IV						
3 year corrected rate	8%		20%		9%	
5 year corrected rate	10%		20		—	

During 1960—1962 some patients were treated with two opposed cobalt 60 beams, in 1964 a cobalt 60 three beam technique with individual dose planning was introduced.

The patients in the second and third groups were selected in that those with conditions more advanced than the average were treated with high energy radiation while those in the first group formed an unselected series. Within the respective clinical stages, the 5- and 3-year survival rates are not strikingly different in the three periods (Table 1). The only tendency towards the superiority of high energy therapy with doses of about 4 500 rad to the parametrium over 5 to 7 weeks, is found in the series of stage III patients receiving individually planned cobalt 60 by the three beam technique. However, the series are small and only 3-year follow-up figures are available.

Several points must be considered as to the factors that may be used by the clinician as a guide in attempts to increase the cure rate. Interest at Radiumhemmet has for example been focused upon the correlation between the histology and prognosis (WETTERDAL 1934) as well as upon the possible prognostic guid-

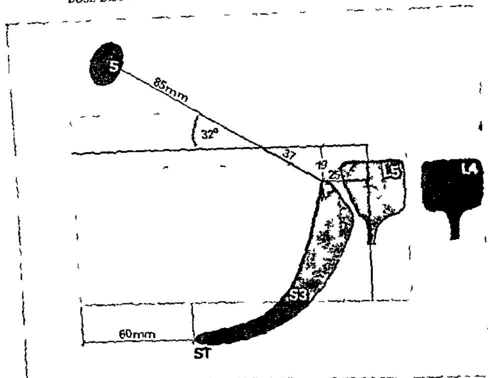


Fig 1 The relationship between the target volume and the pelvic structures The ventral and cranial surfaces of the volume together w th the limits of variation Figures of distances are mean values in mm for the 10 patients under investigation Due to individual differences in the inclination of the p lvis in sup ne position the angle formed by the ventral surface of the target volume a d the line between the *symphysis pubis* (S) and the promontory of the sacrum (P) varied between 23 and 53 mean 3? The mean distance between the caudal surface of the target volume and the tip of *the sacrum* (ST) was 60 mm S3 = middle part of the third vertebra

Supplementary external irradiation Dose planning was performed for external cobalt 60 therapy by the three beam technique developed at Radiumhemmet (RANUDD 1965) The target volume which was to be surrounded by the 95 % isodose curve was carefully determined in each individual instance after thorough clinical examination of the anesthetized patient The extension of the target volume in the ventrodorsal direction was defined by the size of the tumor as estimated bimanually with the addition of 2 cm ventrally and 2 cm dorsally Laterally the target volume was extended to the pelvic wall and in the 10 patients was found to be 16 cm wide The cranial caudal range of the target volume was taken as 16 cm symmetrically distributed around the center of the tumor This was represented in the a p and lateral roentgen films by means

held by a belt (HELANDER & LINDBOM 1955). The a.p. and lateral roentgen films were sometimes supplemented with oblique views.

Thermoluminescence dosimetry. Commercially available detectors, lithium fluoride (LiF) in teflon, 6 mm long and 1 mm in diameter, were introduced between spacers of lead, 7 mm long and 1 mm in diameter, in a teflon catheter, with an outer diameter of 1.8 mm. Each catheter was loaded with 18 detectors.

The teflon catheters were inserted percutaneously into the femoral veins immediately before the radium application under roentgen TV control until the tips lay in the inferior vena cava. They were fixed in position and their location recorded with a.p. and lateral roentgen films. The catheters remained in the veins during the primary radiation treatment of the patient which consisted of combined intra-uterine and intravaginal radium application with the current Stockholm technique (KOTTMEIER 1964).

After removal of the radium from the patient the catheters were withdrawn and the separate detectors were recovered in order and numbered. A partly rebuilt commercial instrument (Con-Rad read-out instrument, model 5100 A) was used for the determination of the dose on each detector, employing its individual calibration factor. Various sources of error can be discriminated and reduced in using thermoluminescence LiF (CARLSSON 1969). The standard deviation varied between 6.6 and 8.7 % of the mean in a study of 10 detectors, treated in the same way as those in the study, and run through eight exposure events.

Dose rate measurement in the bladder and rectum was performed with a Siemens Gammameter by a technique described in detail in a previous paper (JOELSSON & BÄCKSTRÖM 1969). The important features will be repeated.

The centimeter-graduated probe of the Siemens Gammameter was introduced into the urinary bladder and starting 12 to 13 cm cranial to the orifice of the urethra the values of dose rates were noted during its withdrawal. Attention was paid to the mean of the three highest consecutive measurement values at centimeter intervals. Similar measurements in relation to the anal sphincter were performed in the rectum.

All the measurements were made with the patient supine immediately after the application of the irradiators. They were repeated at the end of the treatment immediately before removal of the radium.

The mean of the values determined at the beginning and at the end of the treatment were used as the definitive. The fact that the Siemens Gammameter was calibrated at room temperature but used at body temperature was considered only when dose rates in bladder and rectum were related to doses measured by LiF dosimetry. The observation that the reading of the instrument was lowered by 0.7 % per degree centigrade in temperature was made recently (JOELSSON & BÄCKSTRÖM 1969). Uncorrected figures have been used earlier and have also been correlated to frequency of complications (KOTTMEIER 1964).

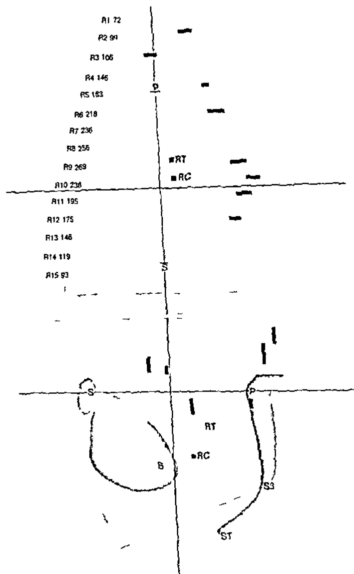


Fig 3 Stage I b Endocervical carcinoma 61 mg Ra 40 mm active length in uterus Flat box 71 mg Ra in vagina Treatment time 25 hours Bladder dose 2 110 rad rectal dose 1 730 rad

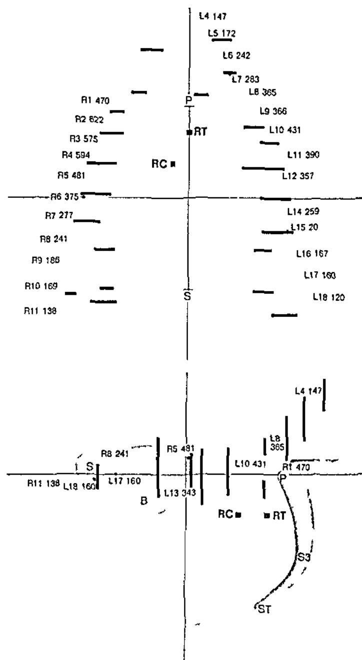


Fig 2 Stage I b Exophytic carcinoma The intra uterine irradiator contains 61 mg Ra (active length 40 mm) and the vaginal flat box 71 mg Ra Treatment time 24 hours Dose at the posterior wall of the bladder 2 030 rad at the anterior wall of the rectum 2 260 rad

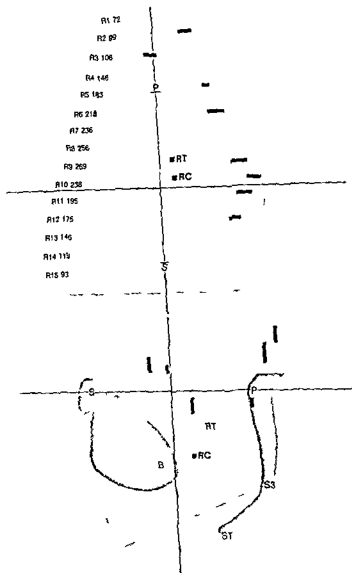


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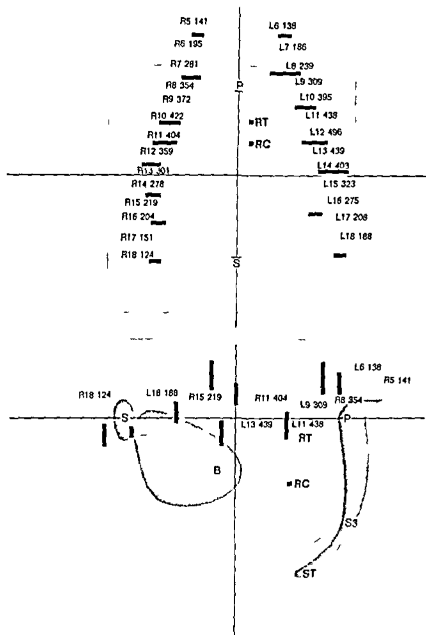


Fig 4 Stage I b Exophytic carcinoma 43 + 54 mg Ra intra uterine tandem 16 + 27 mm active length 71 mg Ra in flat box in vagina Treatment time 25 hours 2 090 rad to the bladder 1 790 rad to the rectum

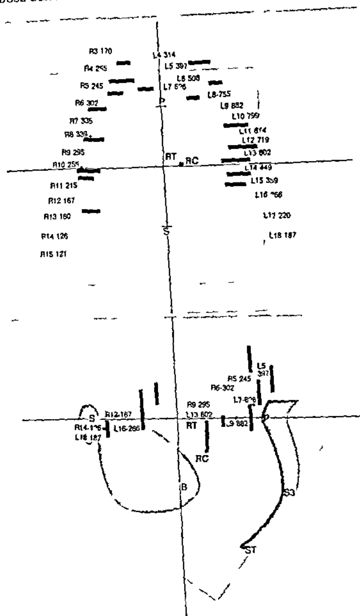


Fig. 5 Stage I b Endo ervical carcinoma 68 mg Ra 45 mm active length in uterus Flat box 71 mg Ra in agina Treatment time 28 hours 2 ?00 rad to the bladder and 1 640 rad to the rectum

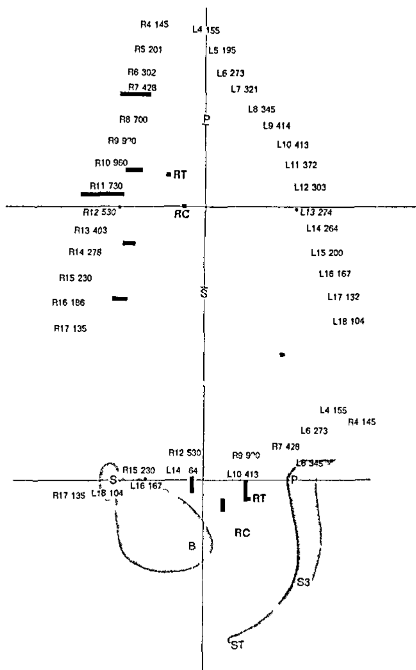


Fig 6 Stage I b *Endocervical carcinoma* *68 mg* Ra active length 45 mm in uterus 71 mg Ra in flat box in vagina Treatment time 22 hours 2 410 rad to the bladder and 2 180 rad to the rectum

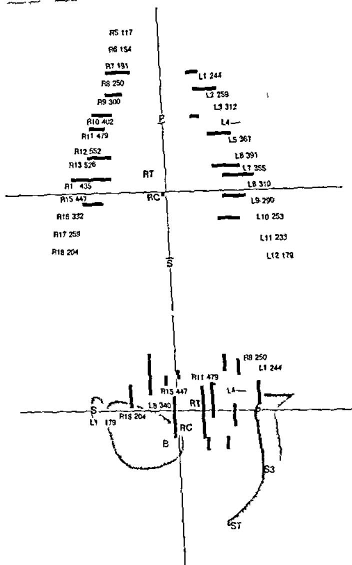


Fig 7 Stage II a Exophytic carcinoma cauliflower type 6? mg Ra active length 39 mm in uterus 80 mg Ra flat box in vagina Treatment time 22 hours ? 380 rad to the bladder and ? 640 rad to the rectum

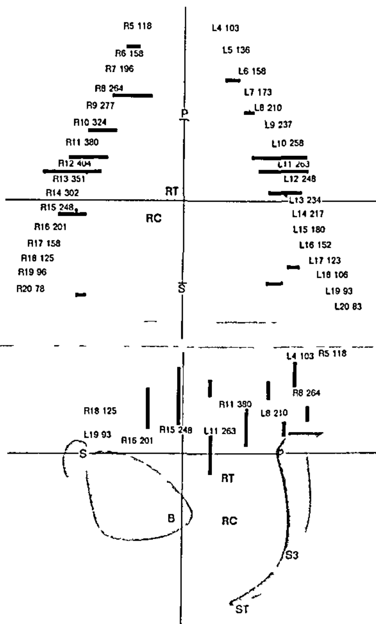


Fig 8 Stage II a Disc shaped carcinoma 70 mg Ra active length 54 mm in uterus 71 mg Ra in flat box in vagina Treatment time 26 hours 1 330 rad to the bladder and 1 940 rad to the rectum

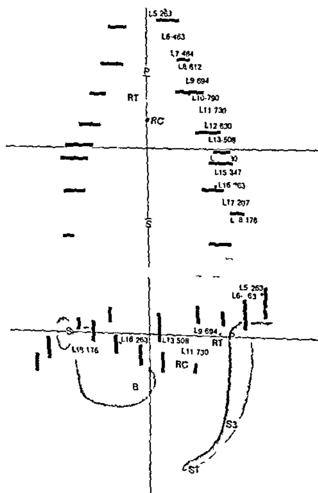


Fig 9 Stage II a Exophytic carcinoma 68 mg Ra active length 45 mm in uterus Flat box mg Ra in vagina Treatment time ?? hours 2 ?80 rad to the bladder and 2 590 rad to the rectum

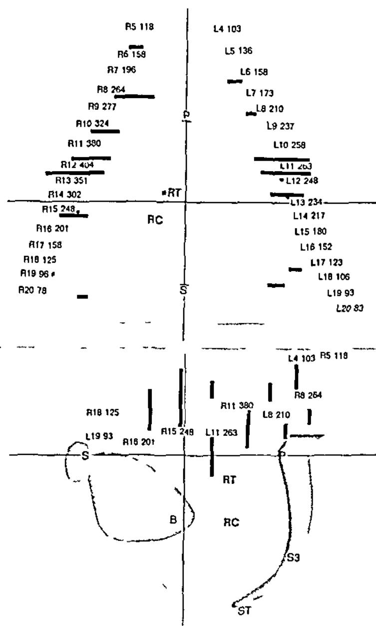


Fig 8 Stage II a Disc shaped carcinoma 70 mg Ra active length 54 mm in uterus 71 mg Ra in flat box in vagina Treatment time 26 hours 1 330 rad to the bladder and 1 940 rad to the rectum

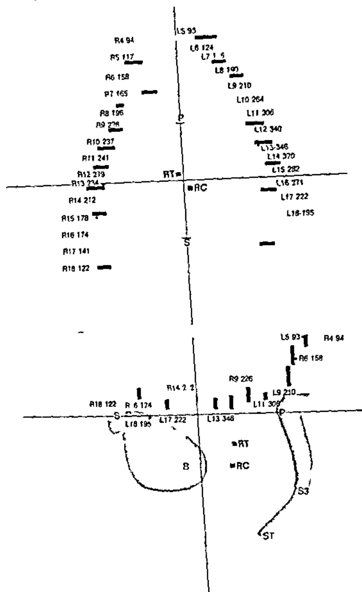


Fig 11 Stage II a Disc shaped carcinoma 62 mg Ra active length 39 mm in uterus 84 mg Ra in flat box in vagina Treatment time 26 hours 1 430 rad to the bladder and 2 020 rad to the rectum

R3 97
R4 106
R5 133
R6 222
R7 282
R8 302
R9 428
R10 496
R11 456
R12
R13 426
R14 370
R15 345
R16 225
RT
RC
P
S
L1 81
L2 156
L3 199
L4 278
L5 300
L6 304
L7 328
L8 337
L9 347
L10 296
L11 284
L1 63
L13 179
L14 145

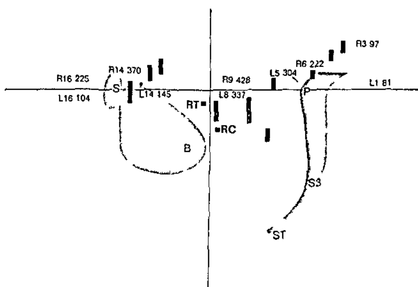


Fig 10 Stage II a Exophytic carcinoma 68 mg Ra active length [illegible] mm in uterus Curved box 77 mg Ra in vagina Treatment time 22 hours 1 800 rad to the bladder and 3 090 rad to the rectum

Table 2

Radiation doses during one course of intracavitary radium treatment to separate parts of the veins in rad as mean values and limits of determinations in ten patients. The values for the cranial and caudal parts of the common and external iliac veins and for the femoral vein are based on measurements of two LiF detectors for each patient. Correction has been made for the contribution from diagnostic roentgen procedures. Data on irradiators used are given in Figs 2 to 11

	Inferior vena cava	Common iliac vein			External iliac vein			Under inguinal ligament
		Cranial A	Middle	Caudal B	Cranial C	Middle	Caudal D	
Right side								
Mean	110	173	195	300	410	473	382	310
Limits	53—171	87—300	116—273	175—468	230—808	239—958	252—628	210—445
Left side								
Mean	146	232	297	381	443	384	360	285
Limits	57—261	95—472	188—508	145—817	190—805	246—567	254—659	203—497

The location of the catheters in some of the patients allowed determinations to be made on detectors in the inferior vena cava and in the middle parts of the common and external iliac veins. The detector exactly posterior to the inguinal ligament was always considered separately. The complete list of determinations is given in Table 2.

Diagnostic roentgen films were taken with the LiF detectors in place. A special study disclosed that the contribution to the thermoluminescent signal from these procedures was 2 to 3 rad. The values in the text and in Table 1 are accordingly corrected while in Figs 2 to 11 the uncorrected dose determinations are given for each specific detector.

The effect on the thermoluminescent detectors from the lead spacers between them in the teflon catheters was investigated in a separate study although no measurable contribution to the signal was anticipated. The results of the measurements indicate no detectable influence on the signal of the detectors from the spacing with lead.

Topography of target volume for supplementary external irradiation. It was a consistant finding that in the a.p. projection the cranial surface of the target volume was located 1 to 4 cm cranial to the promontory (Figs 2 to 11, upper halves). Related to the finding at phlebography the cranial surface of the volume crossed the middle parts of the common iliac veins. The frontal projection of the caudal surface of the target volume in all cases extended caudally to the symphysis pubis by 3 to 5 cm, thus in this view the caudal surface crossed the femoral veins

of an opaque cylinder inserted into the cervical canal. Accurate body contours were drawn in each instance and the target volume in the transverse section recorded according to the above principles.

All the relevant information was transferred from the various roentgen films to one diagram with correction for distortion due to magnification. A line between that part of the symphysis pubis (S) projecting most posteriorly and the promontory of the sacrum (P) was chosen as the main axis of the system of coordinates in the frontal and lateral views. The representation of the lymph nodes, the projection of the centers of the lithium fluoride detectors, (R = right side, L = left side) and the projection of the center (RC) and the top (RT) of the intrauterine irradiator were all referred to this axis (Figs 2 to 11).

The surfaces circumscribing the target volume were indicated as their projection appeared in the frontal and lateral views. Metal frames were taped to the skin of the patient and demonstrated on roentgen film by the orthographic technique. The posterior wall of the urinary bladder (B) was indicated in the lateral view after the instillation of 50 ml Urografin 30 %.

Results

Dose rate measurements in the bladder and rectum. The dose over one course of radium treatment on the posterior wall of the urinary bladder varied between 1 460 rad and 2 650 rad (mean 2 200 rad). The dose to the anterior wall of the rectum amounted to between 1 800 rad and 3 400 rad (mean 2 400 rad).

Thermoluminescence dosimetry in the pelvic veins. The radiation dose to the common and external iliac veins were referred to four divisions (A—D) of the vessels, each 20 mm in length, corresponding to two LiF detectors and one interposed spacer. The right and left sides of the pelvis were considered separately because of the anatomic inequalities. A. In the cranial part of the common iliac vein, the dose varied between 87 and 300 rad on the right side and between 95 and 472 rad on the left side, mean values 173 rad and 232 rad respectively. B. In the caudal portion of the common iliac vein the dose on the right side was 175 to 468 rad, mean 300 rad and on the left side 145 to 817 rad, mean 381 rad. C. In the cranial part of the external iliac veins, distal to the confluence between the internal and external iliac veins the dose on the right side was 230 to 808 rad, mean 410 rad, while on the left side corresponding values were 190 to 805 rad with a mean of 443 rad. D. In the caudal part of the external iliac vein, cranial to the inguinal ligament the dose on the right side ranged between 252 and 628 rad, mean 382 rad, and on the left side between 254 and 659 rad, mean 360 rad.

to the promontory. The cranial surface of the target volume was evident at a distance of 0.7 to 4.5 cm (mean 2.5 cm) cranial to the promontory. The projection of the dorsal surface of the target volume, which was always parallel to the firm support of the patient, usually crossed the sacrum at about the middle of its third vertebra. The caudal surface of the tumor volume was located 2.5 to 8.0 cm (mean 6 cm) caudal to the tip of the sacrum.

It is apparent from the results that the projection of the ventral surface of the target volume crossed the distal part of the common iliac vein or the proximal part of the external iliac vein. The lymph nodes along the external iliac vessels were located outside the target volume in the ventral direction. The target volume enclosed the posterior part of the urinary bladder in all but one patient. Although the rectum was not demonstrated on the roentgen films it was concluded that a part was within the target volume.

Discussion

The fact that survival rates in carcinoma of the cervix uteri are about the same irrespective of differences in techniques should not produce a nihilistic feeling with no attempt being made to improve the results with the facilities now available. The survival rates in early stages of carcinoma of the cervix have already been raised to such a level that a reduction of adverse side effects of treatment may be considered a worth while objective on its own.

High energy therapy makes it possible to deliver a cancericidal dose to the whole pelvis with one or two ventral and dorsal beams, and at Radiumhemmet this technique has been applied in several patients with stage II b and III carcinoma of the uterine cervix. No individual dose planning was performed during the period 1960—1963. Between 2 000 and 5 000 rad (mean 3 700 rad) were delivered over 4 to 5 weeks. An analysis of the patients in stage II b who received cobalt 60 irradiation with two opposed beams in addition to one or two intracavitary applications of radium reveals that the 3- and 5-year survival rates were not statistically different from the figures for those who received conventional roentgen ray irradiation. The same applies to the patients with stage III carcinoma. The differences may be partly explained by the fact that only a small proportion of the total number of patients in stage II b during the time under review were treated with two opposed cobalt beams. In contrast to that in the roentgen ray series almost three fourths of the patients received the specified treatment, which invalidates too close a comparison between survival rate figures (Table 1). It is of interest, however, to find that the frequency of complications from the urinary bladder grade II to III was raised from 6 % (conventional roentgen rays) to 15 % (^{60}Co, two opposed beams) and the

Table 3

Frequency of lymph node involvement (%) in stage I and II carcinoma of the uterine cervix. The patients underwent operation as primary treatment and lymphadenectomy was performed compulsory (From REIFENSTUHL *1967 and the references therein.)*

	Number of patients	Stage	
		I	II
ANTOINE (1959) (FROEWIS & ULM)	544	8 5	27 9
DE BIASI (1954) (PAPADIA)	135	20 7	31 9
BRUNSCHWIG & ROESLER (1957)	74	14 0	34 0
CARTER et coll (1953)	79	16 7	25 9
CATTANEO (1954) (MARZIALE)	66	20 0	—
CHRISTENSEN et coll (1955)	100	21 3	41 0
CURRIE (1962)	339	16 0	38 0
GRAY (1958)	61	11 0	35 0
GUSBERG et coll (1953)	64	10 0	31 0
KIMBROUGH (1959)	84	34 0	51 0
LANGE (1960)	178	28 8	43 8
LIU & MEIGS (1955)	258	17 0	40 0
MARTINEZ et coll (1953)	442	23 0	41 0
MEDINA et coll (1959)	60	20 0	40 0
MEIGS & MORTON (1958)	130	18 0	37 0
MITANI et coll (1957)	182	39 9	33 6
MITANI et coll (1962)	78	30 2	—
MITRA (1959)	192	18 0	29 0
MORTON et coll (1952)	89	15 0	24 0
NAVRATIL (1955)	180	20 0	29 0
PARSON (1962)	80	13 0	22 0
SHERMAN et coll (1952)	176	12 0	29 0
TACHIBANA (1956)	416	12 0	23 7
WELCH et coll (1961)	383	12 0	24 0

below the inguinal ligaments The lateral surfaces of the target volume in every instance enclosed the frontal projection of the lymph nodes although the margin was small

In the lateral view the intersection between the projection of the ventral surface of the target volume and the line connecting the promontory of the sacrum with the symphysis pubis was located a sixth to a half (mean a third) of its length from the promontory (Fig 1 and Figs 2 to 11, lower halves) The angle between the projection of the ventral surface of the target volume and the conjugate diameter varied between 23° and 53° (mean 32°) The lateral projection of the ventral surface of the target volume was 0 8 to 3 7 cm (mean 1 9 cm) ventral

tissue It is reasonable to postulate that a modification of the technique of external irradiation to include the lymph nodes along the external iliac vessels in the target volume would be to the advantage of the patient and lead to an improvement in the survival rates Changes have therefore been considered along different lines The simplest modification implies an increase in the load on the ventral beam by a factor of two or more If high energy electrons are available another choice will be two frontal electron beams as a supplement to the original three beam technique The electron portals can be designed so that they obliquely transverse the frontal cobalt 60 field and cover the lymph node chain only The energy of the electrons must be adapted to the individual patient within the limits of 15 to 25 MeV

SUMMARY

A series of patients examined with lymphography phlebography LiF dosimetry and localization of the target volume disclosed that the pelvic wall dose from intracavitary treatment was small and that the three beam technique for external cobalt irradiation failed to include the lymph nodes along the external iliac vessels This finding necessitates a modification of the technique A redistribution of the loads of the three beams or the ddition of hi h energy electron beams are suggested

ZUSAMMENFASSUNG

Es zeigte sich bei der Untersuchung von einer Serie von Patienten mittels Lymphographie Phlebographie LiF Dosimetrie und nach Messung der Tiefendose dass die Dosis zur Wand der Pelvis bei intrakavitarer Behandlung niedrig war und dass die Dreifelderbestrahlung mit der Kobaltbombe die Lymphknotengrupp entlang der A iliaca externa nicht genugend erfast Hieraus ergiebt sich dass eine Aband rung der Technik notig ist Eine Neuverteilung der Felder i t notwendig oder die Zugabe von zusatzlichen Feldern von hochgeschwindigen Elektronenstrahlen

RÉSUMÉ

Les examens au moyen de lymphographie phlebographie dosimetrie par LiF et la localisation du volume cible chez un s rie de malades atteintes de cancer du col de l uterus ont montre que la dose à la paroi du bassin est petite et que la technique d irradiation externe au cobalt par trois champs n inclue pas les ganglions lymphatiques situes le long des vaisseaux iliaques externes Cette con tatation impose une modification de la technique Les auteurs proposent une repartition differente des doses faites par les trois champs ou l addition d une irradiation par des electrons d haute energie

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frequency of rectal complications grade II to IV from 8 % to 20 % (For the grading system the reader is referred to KOTTMEIER 1964)

It was hoped that a more accurate administration of the dose to the tumor area and especially to the internal iliac and common iliac lymph nodes with the three beam technique established in recent years might improve the results without causing damage to normal tissue This technique in combination with individual dose planning and absorbers of special size and shape in the frontal beam during a number of the fractions also reduces the dose to the anterior part of the bladder and posterior part of the rectum Only three year figures are available at the present time and even if these do not consistantly indicate any superiority of the technique as regards survival, a clear tendency towards a decreased incidence of complications exists in spite of an average dose of 4 500 rad (2 500 to 6 700 rad) over 5 to 7 weeks

The routes of spread of carcinoma of the cervix are into the vaginal mucosa, into the myometrium of the lower uterine segment and into the network of lymphatics of the paravaginal as well as the paracervical tissues Combined statistics in the literature present figures of involvement of the nodes at the pelvic wall of between 15 and 20 % in stage I carcinoma, and 30 to 35 % in stage II carcinoma (Table 3) The variation in the figures are to some extent dependent upon whether single or serial sections of the lymph nodes have been studied A considerable increase in lymph node involvement has been observed by some authors when serial sections have been examined (LANGE 1960, AHRENS & TSCHOKE 1961, HUHN 1962 and MITANI et coll 1962) It is known that a considerable proportion of involved nodes are to be found along the external iliac vessels (cf REIFFENSTUHL 1967) The doses to the common and the external iliac vein are comparatively low with intracavitary radiation methods and compared to the doses to the posterior wall of the bladder and the anterior wall of the rectum the doses at the pelvic wall are five to eight times smaller Doses of similar magnitude measured both by intravascular thermoluminescence detectors (TJERNBERG et coll 1968) and with an ionization chamber during the procedure of extraperitoneal lymphadenectomy (KOTTMEIER 1951) have been reported earlier The correlation between isodose rates around the combined intra uterine and vaginal irradiators and pelvic anatomy reported by WALSTAM (1954) are also in agreement with the observations in the present study

The magnitude of the supplementary dose to be given to the parametrium by external irradiation is determined in each instance by the stage of the disease and condition of the patient The three beam technique produces steep dose rate gradients towards the surrounding tissue, and necessitates the inclusion of relevant tissues in the target volume The observation is described in this paper that the routine three beam technique may well exclude some potentially malignant

tissue It is reasonable to postulate that a modification of the technique of external irradiation to include the lymph nodes along the external iliac vessels in the target volume would be to the advantage of the patient and lead to an improvement in the survival rates Changes have therefore been considered along different lines The simplest modification implies an increase in the load on the ventral beam by a factor of two or more If high energy electrons are available another choice will be two frontal electron beams as a supplement to the original three beam technique The electron portals can be designed so that they obliquely transverse the frontal cobalt 60 field and cover the lymph node chain only The energy of the electrons must be adapted to the individual patient within the limits of 15 to 25 MeV

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FROM THE NATIONAL INSTITUTE OF RADIATION PROTECTION THE DEPARTMENT OF CLINICAL BIOCHEMISTRY ROYAL VETERINARY COLLEGE, AND THE RESEARCH INSTITUTE OF NATIONAL DEFENCE STOCKHOLM SWEDEN

WHOLE BODY MEASUREMENTS ON THE DISTRIBUTION OF MERCURY-203 IN HUMANS AFTER ORAL INTAKE OF METHYLRADIOMERCURY NITRATE

by

R FALK J O SNIHS L EKMAN U GREITZ and B ÅBERG

The presence of mercury contaminants in nature has been proven in the past few years mainly in fresh water fish Since as much as 12 mg/kg (wet weight) of mercury has been reported in Sweden it must be assumed that the lower consumption of fish is the reason for the avoidance of such mercury poisoning as have occurred in Minimata and Nigatu in Japan Sale of fish containing more than 1 mg of mercury per kg wet weight is now forbidden in Sweden

Irrespective of the chemical form of the mercury contained in the effluents methyl mercury is the ultimate product form after the process of methylation occurring in nature In order to study in more detail the uptake and metabolism of methyl mercury in the human three male volunteers 37 42 and 44 years of age each took 2 6 μCi of monomethyl 203 mercury nitrate orally Measurements were made for a period of more than two months on urine and faeces and for more than seven months on total body activity The result of the whole study has been presented by ÅBERG et coll (1969) The purpose of the present report is

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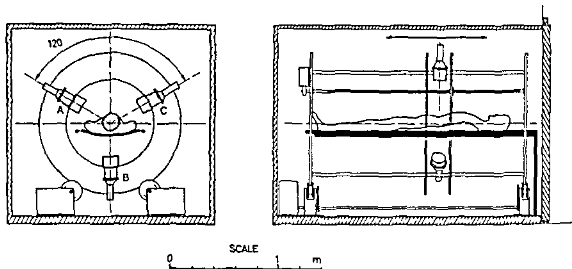


Fig 1 The three crystal counter

to give a detailed description of the distribution measurements with the whole body counter employed Results of the distribution measurements are given, as well as a description of the methods used for measurement, calibration and sources of error The report should thus give a picture of the possibilities provided,

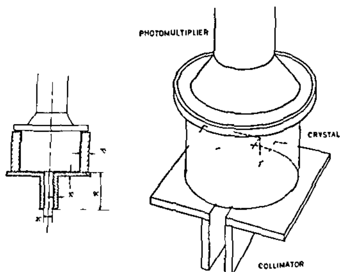


Fig 2 The collimator arrangement

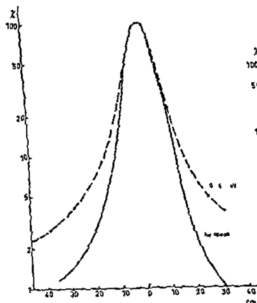


Fig 3 Profile curves obtained from a ^{203}Hg source with different energy intervals

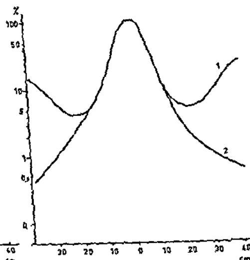


Fig 4 Comparison of the resolution with the present (2) and the previous (1) collimator arrangement

as well as the limitations of the whole body counter technique for similar investigations

Counters and collimators The measurements were carried out with the three crystal counting apparatus in the low background laboratory of the National Institute of Radiation Protection Stockholm The apparatus has been described in detail previously (LINDELL & MAGI 1966 MAGI 1967) The human subjects to be measured were in a supine position with the crystals placed symmetrically around them For each measurement the crystals were moved axially with respect to the length of the body at least 2 cm (step scanning) (Fig 1) Each crystal Ø 5 × 4 NaI(Tl) was provided with a simple slit collimator of lead having a thickness of 10 mm and a height of 90 mm (Fig 2) Each measurement was run for at least 3 minutes The crystals were connected to a pulse height analyzer with a magnetic tape recording device

For the drawing of the profile curve the *net counts* per minute in channels 22—31 corresponding to the energy interval 220—320 keV were used (^{203}Hg decays to 82 % via 279 keV γ radiation) In this way the resolution of the profile

curve was better than if the entire energy interval, 0—4 000 keV, had been used (Fig 3) This improvement in the resolution did not adversely affect the statistics

During the period of the investigation (14 weeks), the collimators were in the process of improvement The resolution for the two collimations used appears in Fig 4, the less good of the two was used in the initial phase of the series of measurements (EKMAN, GREITZ, MACI et coll 1968) but was found to give an insufficient resolution for the results sought This part of the measurements has therefore not been taken into account in the presentation and discussion of the results

Background measurements No opportunity was provided for making whole-body measurements of the subjects prior to the intake of the ^{203}Hg To provide the necessary background count, a 4th subject whose weight and length correspond to the average of the three subjects, was measured in the whole body counter A summary of the gross counts (subject plus apparatus background) within the energy interval used for 12 subjects registering activity only from ^{137}Cs (from fall out) and natural activity (mainly ^{40}K), is given below

Subject	Gross counts in cpm
1	448
2	406
3	423
4	406
5 (subject used as background)	465
6	405
7	419
8	395
9	428
10	410
11	406
12	471
	424±24

These measurements were made by scanning the length of the body without collimation, and therefore give an indication of the distribution of the background among the 12 subjects The activity of the subject used as background is marked in the table, showing that his activity was higher than the average (though within $\pm 2\sigma$) The result of the measurement of the profile for this subject is shown in Fig 5 Collimators were used for this measurement The background used is the average of the profile, which implies that the net values measured on

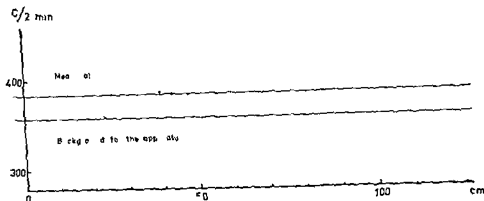


Fig 5 Profile curve for a background subject

the subjects are somewhat low in the area of the head and high in the trunk region on the assumption that the background used is the true background This consideration has a certain importance for the accuracy of the final measurements that are discussed below

Definition of the regions The aim of the measurements was to study the relative distribution and possible re distribution of the activity in the human body For this purpose the profile curve was divided into five regions including the following organs region (1) brain (2) thyroid (3) liver and spleen, (4) bladder and (5) thigh muscles

As the division was made with respect to the anatomical details the linear extention of the regions were not equal

Region boundaries	A		B		C		D		E		F
Regions		1		2		3		4		5	

(A) skull cap (B) a plane below tragus at 1/4 of the distance between tragus and jugular notch (C) jugular notch (D) umbilicus (E) ischial tuberosity and (F) knee

Identification of the organs As can be seen from the profile curve (see Fig 6) two marked peaks appear one in region (1) and the other in region (3) Attempts were made in three different ways to identify the organs in these regions

1 *Determination of the linear size of the organ* (parallel to the length of the body) corresponding to the peaks in the profile curve Profile curves were taken of samples with various linear sizes to determine the FWHM (the full width of

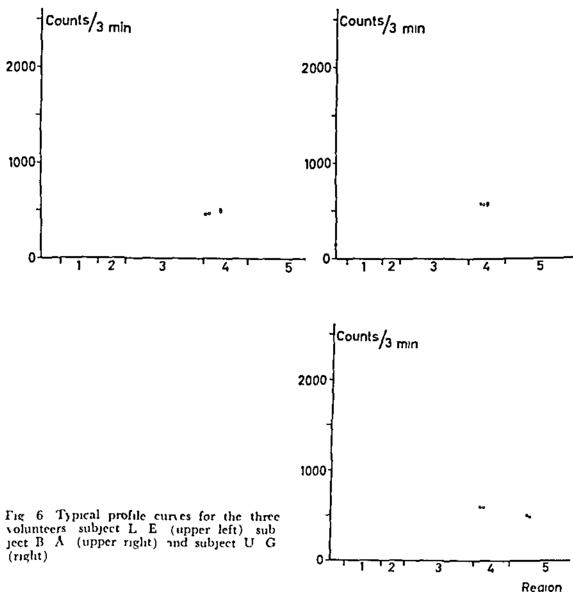


Fig 6 Typical profile curves for the three volunteers subject L E (upper left) subject B A (upper right) and subject U G (right)

half maximum) (see Fig 7) Thus the size of the organ can be determined using the relationship $d_0 <$ size of the organ $< d_1$ where $d_0 > 0$ For the peak in region (1) the size of the organ was determined to be < 15 cm and for the one in region (3) 10 cm $<$ size of the organ < 20 cm

2 *Determination of the lateral position* was achieved by making measurements with one crystal at a time, the net counts indicating the asymmetrical position of the organ The results of the measurements in region (3) using the experi

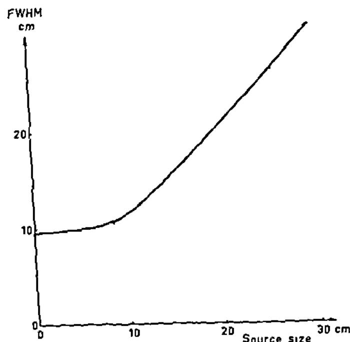


Fig 7 Full width of the half maximum of the profile curve as a function of source size

mental set up shown in Fig 1 were as follows with the counting rates in per cent

	Crystal A	Crystal B	Crystal C
Subject L E	23	37	40
Subject U G	20	37	43

The results indicate that the organ is situated on the right hand side of the body

3 *Determination of the longitudinal position* requires measurement of the profile It can be seen from Fig 6 that the position of the organ in region (1) lies in a plane perpendicular to the length of the body and through the eyes and the occiput of the skull

These three observations lead to the conclusions (A) that the major part of the activity in region (1) is concentrated within a small sub-region the position of which indicates that it may be the cerebellum and (B) that the major part of the activity in region (3) appears to be accumulated *in the liver In the other* regions no specific site of the activity can be deduced

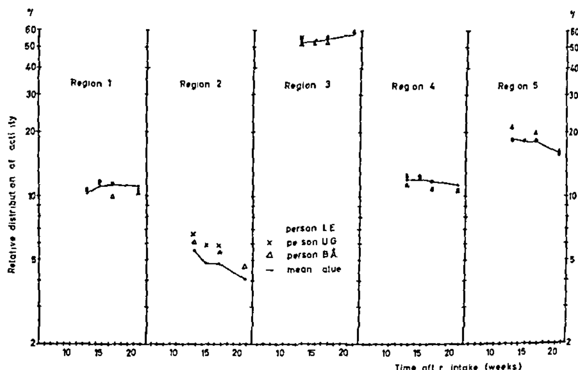


Fig 8 Relative distribution of activity in different regions as a function of time The figures are not corrected for systematic errors

Distribution of activity, accumulation and biologic half lives

A profile curve of net counts within the peak was drawn on a linear scale for each subject and measurement After marking the five regions, the areas under the curves were cut out and weighed In this way the weight represented the total counts within each region The results are shown in Fig 8 The following *conclusions may be drawn*

1 Deviations among the three subjects were small and it seems probable that they can be regarded as non significant when the errors (discussed below) have been taken into account The average of the three subjects has been taken for each measurement and connected in the diagram by a line

2 The lines indicate a gradual accumulation of the activity in regions (1) and (3), corresponding to the cerebellum and the liver This is compensated for by a relative decrease in the other regions as can be seen from Fig 8 The most rapid depletion appears to take place from region (2) (thyroid) The uncertainty in this region is, however, relatively large (see below)

The same result can be seen in Table 1 which summarizes the biologic half lives Results have been included which have been calculated from the data

Table 1

Summary of the biologic half lives in days

Subject	Regions						
	Previous experimental set up	New experimental set up					
	1—5	1—5	1	2	3	4	5
L E	61	69	95	36	72	53	57
U G	75	70	95	48	68	73	68
B Å	78	60	64	45	73	54	49
Mean	71	66	85	43	71	60	58

The half l fe for region (2) is p obably somewhat underestimated because of the systematic errors d scussed below

Table 2

Distribution in per cent of ^{203}Hg — The total activity in regions 1—5 is set to 100

Subject	Date	Regions				
		1	2	3	4	5
L E	26/3	9.7	4.1	54.0	13.0	19.2
	8/4	10.4	3.3	54.2	13.2	18.9
	22/4	10.4	3.1	55.2	12.2	19.1
	22/5	11.1	2.5	58.6	11.3	16.5
U G	27/3	8.8	6.7	54.4	13.1	17.0
	9/4	9.5	6.0	53.8	12.8	17.9
	23/4	11.0	6.0	52.4	13.5	17.1
	20/5	9.5	5.3	55.1	13.5	16.6
B Å	25/3	9.3	6.3	50.5	11.9	22.0
	10/4	10.6	5.6	51.5	12.0	20.3
	24/4	8.9	5.6	53.5	11.3	20.7
	29 5	9.2	4.7	58.2	11.1	16.8

obtained in measurements using the previous experimental set up with the less satisfactory collimators This fact does not impair the result for this case

The half lives have been determined from diagrams in which counts corrected for radioactive decay are plotted versus time for each region (see Fig 9) The biologic depletion has been assumed to occur at the rate represented by the

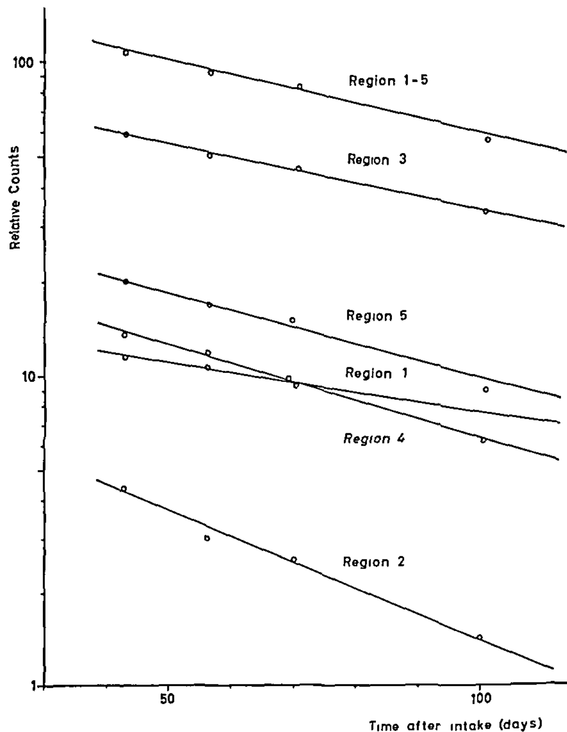


Fig 9 Determination of the biologic half life of methyl [203]Hg for subject I F

Table 3

Distribution of ^{203}Hg *in nCi*

Subject	Date	Regions					
		1	2	3	4	5	1—5
L E	26/3	90	40	500	120	180	970
	8/4	70	20	350	90	120	650
	22/4	50	10	260	60	90	470
	22/5	20	5	120	20	30	210
U G	27/3	80	60	470	110	150	860
	9/4	60	40	350	80	120	640
	23/4	50	30	220	60	70	430
	20/5	20	10	120	30	40	220
B A	25/3	90	60	510	120	220	1 000
	10/4	70	40	330	80	170	640
	24/4	40	30	240	50	90	450
	29/5	20	10	100	20	30	180

straight line drawn in the figure which corresponds to a one phase exponential process

To obtain an indication of the absolute distribution of the activity the number of counts must be corrected for absorption The following assumptions have been made concerning the activity in the various regions, taking into account the previous identification of the organs (1) all activity in the cerebellum (2) activity evenly distributed (3) all activity in the liver (4) activity evenly distributed (5) activity evenly distributed

By making measurements with water phantoms the relationship between number of counts and activity was determined for the five regions The activity was either evenly distributed throughout the phantom corresponding to regions (2) (4) and (5) or concentrated as in regions (1) and (3) The correction factor is expressed in nCi/mg from which after multiplication with the weight of the area under the profile curve, the activity is obtained in nCi The correction factors for the five regions were for (1) 0 76 for (2) 0 86 for (3) 0 84 for (4) and (5) 0 88 nCi/mg The distribution of the activity is shown in Table 2 In Table 3 the distribution of the activity is expressed in nCi

Comparison between the measured and the expected total activity As has been pointed out in the introduction the three subjects took an amount of ^{203}Hg

Table 4

The statistical counting error in subject L E at different measurement dates

Date	Region	Per cent	Date	Region	Per cent
26/3	1	2	22/5	1	5
	2	4		2	25
	3	1		3	2
	4	2		4	8
	5	3		5	8

orally on the 12 February 1968, which was measured to be 3 0 μCi on the 2 February 1968

Measurements on urine and faeces (EKMAN, GREITZ, PERSSON & ÅBERG 1968) on the days immediately following the intake, showed that the uptake was nearly 100 % Based on the data of the distribution of the activity on the 25—27 March, and on the biologic half life which was found with the previous collimation, (see Table 1), the total activity was determined for the 2 February 1968 The result for subject L E was 3 6 μCi, for U G 3 1 μCi and for B Å 3 4 μCi

The correspondence must be regarded as satisfactory when consideration is given to the simplicity of the phantom models used in the comparison measurements, and that the experiments a priori were not set up to be absolute measurements

Sources of error and their influence on the results

I *Counting statistics* The statistical counting errors increased as the experiments progressed, since the decrease in the activity could not in practice be compensated for by correspondingly longer measurements Table 4 shows the error in per cent in the five regions for the first and last measurements in subject L E

II *Changes in position* It was difficult to avoid slight movement of the subjects during the course of the relatively long measurements Thus, longitudinal shifts of some centimetres could occur Significant shifts in position were checked by a measurement of the distance between the top of the head and the top of the liver Nevertheless movements in one or other direction involve an error of ⩽ 4 %

III *Background* The fact that the correct background for each subject could not be used could have introduced an error which would be largest in the final

Table 5

Errors in per cent of the distribution percentage for the five regions at different measurement dates

Date	Region	Random error (%)	Systematic error (%)	Systematic error (%) when error VII=0
26/3	1	5	+16	+7
	2	6	−24	−30
	3	5	−6±2	−7±2
	4	5	−14±[illegible]	−10±2
	5	5	−11±[illegible]	−7±2
22/5	1	7	+25	+16
	1	5	+4	+8
	3	5	−6±7	−7±7
	4	9	−14±4	−10±4
	5	9	−11±8	−7±8

measurements in the series To be able to obtain an estimate of the size of this error the activity distribution was calculated with three different background values (1) background constant over all the five regions (2) different background for each region corresponding to a background profile, and (3) different background for each region corresponding to the background profile for the subject having the lowest background (see tabulated data p 58) The conclusions were the following The scatter of the three alternative values for the different regions was largest in the final measurement The absolute differences between the highest and the lowest values were for region (1) = 1 4 % (2) = = 0 6 % (3) = 3 6 % (4) = 0 5 % and for (5) = 1 1 % Irrespective of which background value was used, the same tendency to increase or decrease is noted in the various regions

IV *Influence of adjacent regions* Since the crystals see a larger field than that corresponding to the width of the slit a certain overlap of adjacent regions occurred The most serious overlap occurs in region (2) from activity in region (3) Region (2) was therefore studied in subject L E who was the shortest of the three and in whose case the influence between the regions can be expected to be the greatest Using a ^{203}Hg source of suitable size a profile which corresponded to the top of region (3) was measured From this the influence on region (2) was calculated The practical result was that approximately 10 % of the number of counts measured in region (2) are due to activity actually in region (3) In the other regions the mutual influence was of little significance

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The statistical counting error in subject L F at different measurement dates

Date	Region	Per cent	Date	Region	Per cent
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	2	4		2	25
	3	1		3	2
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	5	3		5	8

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III *Background* The fact that the correct background for each subject could not be used could have introduced an error which would be largest in the final

estimate them The error discussed under III is systematic in character but could not be estimated for each subject The possible deviation was estimated by calculations the results of which are given under III To determine the sign of the error for each subject, the ratio peak/total net counts was calculated for each region in the first and final measurements This ratio should remain constant if the correct background had been used However it is seen that the ratio peak/total counts is clearly larger for the final measurements than for the first for regions (1) and (2) thus giving the error a positive sign, i e the correct value is higher than the measured value For the remaining regions this significant difference is not noted, thus giving either positive or negative errors In contrast to the other systematic errors (see below) error III increases with time Error V is obviously systematic and implies that region (1) was weighted too lightly, while the others were weighted too heavily Finally, error VII is systematic The percentage errors of the distribution per cent in the five regions for the first and the final measurements are summarized in Table 5

It should be pointed out that no results in the text the tables or the figures have been corrected for the systematic errors If the total activity is corrected for the resulting maximum systematic errors a higher value for the calculated activity intake in μCi is found As is seen above this value is already somewhat too high even without this correction Concerning the biologic half lives Fig 9 and Table 1 and the changes in relative distribution of activity in the five regions (see Fig 8) the systematic errors in IV V and VII have no significant influence on the results If a correction is made for all the systematic errors the percentage distribution of the activity in the five regions is changed as follows an increase in region (1) of 1—3 % (absolute %) in region (3) of 0—2 % while the changes in the other regions are less Relatively the systematic correction is large in region (2) at 25 % and in region (1) at 15 %—20 % A major part of the systematic error originated from the use of an incorrect background A further source of error which could be decreased was the collimation The collimators used cut off a large part of the detector areas leading to impaired sensitivity With focussing slit collimators both the sensitivity and the resolution could be improved

Resulting radiation doses and ethical problems The resulting doses of radiation were calculated for the following organs and on the basis of the points below

1 Intake 3 μCi ^{203}Hg
2 100 % uptake
3 10 % in the cerebellum 54 % in the liver
   36 % in the other parts of the body
4 Biologic half life 85 days for the cerebellum

V *The division into regions* Due to practical considerations, the profile scanning could not be carried out along the entire length of the body, and had to be limited to the area from the top of the head to the knee Thus the regions beyond the skull cap and below the knee were not included If these regions are extrapolated on the basis of the most feasible extensions of the measured profiles, an alternative percentage distribution will be found For the region including the brain, which was studied from this point of view experimentally, an increase in the percentage of activity of only 0 5 % was found (absolute %) With the boundary lines used, over 90 % of the total body activity was covered, and approximately 90 % of the activity in the region including the brain

VI *Cutting and weighing errors* As mentioned above, the profile curves of the net counts within the ^{203}Hg total absorption peak were drawn on linear diagrams, cut out and weighed The random errors introduced by this process were determined to be at a maximum 2 %

VII *Absorption effects* Possible absorption effects were studied by drawing profile curves from measurements on phantoms consisting of ^{203}Hg solutions of varying geometry and homogeneity The result is expressed in nCi/mg (see above), or in mg/nCi, indicating the sensitivity It could thus be seen that, using a phantom corresponding to the head (region 1), the sensitivity was increased by 13 % when the activity was concentrated in the cerebellum, as compared to when it was homogeneously distributed With phantoms corresponding to the trunk, the sensitivity was increased by 5 % when the activity was localized at the position of the liver, as compared to a homogeneous distribution For the calculation of the activity in the five regions in the subjects measured, we have, as mentioned above, assumed that the activity was concentrated in the cerebellum and the liver, in regions (*1*) and (*3*), respectively, and homogeneously distributed in the other regions It may be that a systematic error was thus introduced due to the possibility that there may have been some activity in regions (1) and (3) not concentrated in the organs mentioned The greatest error thus introduced can be calculated by assuming a homogeneous distribution In this case the activity is increased by 13 % in region (1) and by 5 % in region (3) The percentage distribution of the activity is thus changed such that the percentage in region (1) is increased by 9 %, in region (2) decreased by 4 % in region (3) increased by 10 % and in regions (4) and (5) decreased by 4 % (relative %)

Discussion

Comments on the resulting errors Of the errors discussed above those under

estimate them The error discussed under III is systematic in character but could not be estimated for each subject The possible deviation was estimated by calculations the results of which are given under III To determine the sign of the error for each subject the ratio peak/total net counts was calculated for each region in the first and final measurements This ratio should remain constant if the correct background had been used However it is seen that the ratio peak/total counts is clearly larger for the final measurements than for the first for regions (1) and (2) thus giving the error a positive sign, i e the correct value is higher than the measured value For the remaining regions this significant difference is not noted thus giving either positive or negative errors In contrast to the other systematic errors (see below) error III increases with time Error V is obviously systematic and implies that region (1) was weighted too lightly while the others were weighted too heavily Finally error VII is systematic The percentage errors of the distribution per cent in the five regions for the first and the final measurements are summarized in Table 5

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Resulting radiation doses and ethical problems The resulting doses of radiation were calculated for the following organs and on the basis of the points below

1 Intake 3 μCi ^{3}Hg
2 100 % uptake
3 10 % in the cerebellum 54 % in the liver
 36 % in the other parts of the body
4 Biologic half life 85 days for the cerebellum

and 71 days for the other organs

5 Weight of cerebellum 150 g, of the liver 1 700 g and of the whole body 70 kg

The resulting doses were therefore, 600 mrad to the cerebellum, 300 mrad to the liver and 6 mrad to the rest of the body If the activity had been distributed in other parts of the brain, then the dose to the cerebellum would have been less and similarly for the liver Thus the doses estimated for the cerebellum and the liver are expected to be the maximum possible doses

The experiments were carried out on three research workers who have detailed knowledge of isotope work and were thus able to judge both the radiation and the toxicity risks involved in the experiments Therefore no ethical radiation problems arose in this case In other cases, however, special attention must be paid to such problems According to normal practice in Sweden, a subject must actively volunteer, and further, have the understanding and the background to be able to assess the possible risks to which he may be exposing himself At several of the larger hospitals isotope committees have been set up in order to facilitate the appraisal of these risks Each investigation in which human subjects are used, even such studies which can be classed as non routine diagnosis or therapeutic treatment, or which are undertaken not only for the good of the subject (patient) have to be appraised and approved of by such a committee As a general rule, total doses which are less than the permitted weekly dose for personnel engaged in radiologic work according to the ICRP, are considered to be insignificant from the point of view of health risks

Effects noted during calibration For calibration, phantoms were used which consisted of a number of 500 ml plastic bottles containing a solution of methyl ^{203}Hg In order that the bottles should be interchangeable, the quantities contained in them were accurately equal This was assured partly by accurate pipetting and also by measuring the radioactivity in each bottle However, it was noted that after a certain period of time, the amount of ^{203}Hg in the bottles varied, and after further check measurements it could be shown that, when the radioactive decay had been corrected for, the amount of ^{203}Hg had decreased by a half after approximately 4 months As leakage of the solution could be discounted, the only feasible explanation is that methyl mercury diffuses through the polyethylene walls of the bottles

The effect observed here can have serious consequences if, for example, polyethylene bottles are used to contain standard samples of methyl ^{203}Hg Further, there is considerable risk of contamination of adjacent areas and personnel It may be mentioned that inorganic mercury in solution does not diffuse through the walls of polyethylene containers

Acknowledgements

This work has been carried out at the National Institute of Radiation Protection Stockholm and the authors acknowledge humbly their indebtedness to the director Professor Bo Lindell for his interest and support The authors are greatly indepted to Mr Gunnar Eklund who has performed the measurements and given valuable assistance in the work

SUMMARY

Distribution measurements were made using a whole body counter on three subjects who had taken 2 6 μCi CH_3 ^{203}Hg NO_3 orally Profile curves showed that the uptake was specifically concentrated to two regions in the body which correspond to the brain and the liver The biologic half life was calculated The various sources of error are discussed and conclusions drawn concerning their effect on the final results. The radiation doses were estimated and were found to be acceptable The results reported concern specifically the three subjects involved in the present series of measurements but it may be assumed that these results are also applicable to other clinically healthy men 35 to 45 years of age

ZUSAMMENFASSUNG

Bei drei Personen die 2 6 μCi CH_3 ^{203}Hg NO_3 oral eingenommen hatten wurde die Kopenverteilung unter Anwendung eines Ganzkorper Zahlers bestimmt Aus den Profilkurven geht hervor dass die Aktivitat spezifisch in zwei Korperregionen und zwar dem Gehirn und der Leber konzentriert wird Die biologische Halbwertszeit wurde berechnet Die verschiedenen Fehlerquellen werden diskutiert und die Folgerungen daraus fur die Endresultate gezogen Es wurden die Bestrahlungsdosen bestimmt die sich als acceptabel erwiesen Die mitgeteilten Resultate gelten fur die drei Personen der vorliegenden Mess Serie aber es kann angenommen werden dass diese Ergebnisse auch fur andere klinisch gesunde 35 bis 45 jahrige Manner gultig sind

RÉSUMÉ

Des mesures de distribution de dose ont éte faites au moyen d un compteur corporel total sur trois sujets qui avaient pris par voie bucale 2 6 μCi CH_3 ^{203}Hg NO_3 Les courbes de profil ont montre que la fixation était concentre selectivement dans deux regions du corps qui correspondent au cerveau et au foie Les auteurs ont calcule la demi vie biologique Ils examinent les differentes causes d erreur et tirent des conclusions concernant leur influence sur le resultat final Ils ont estime les doses de radiation et les ont trouvees acceptables Les resultats presentes concernent specifiquement les trois sujets soumis à cette serie de mesures mais on peut supposer que ces resultats sont aussi applicables à d autres hommes en bonne sante âges de 35 à 45 ans.

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FROM THE CITY OF HOPE NATIONAL MEDICAL CENTER, DUARTE, CALIF U S A

HIGH ENERGY ELECTRON BEAM TREATMENT PLANNING FOR BETATRON 10—24 MeV

by

RICHARD F NELSON

Historically the first successful operation of the betatron was accomplished by KERST at the University of Illinois in 1940 Earlier attempts were made by WIDERO of Germany WALTON in England and BREIT & TUVE in the United States Although the concept of the equilibrium orbit was known before KERST s work the careful theoretical study of the conditions for particle injection made by KERST & SERBER resulted in making the betatron a practical instrument

In 1945 KERST proposed a method for extracting electrons from the betatron using a magnetic shunt Using this method HARVEY HASS & LAUGHLIN (1951) extracted electrons from the betatron at the University of Illinois Medical School and proceeded with their clinical application The principles of high energy electron beam treatment planning were then initiated and developed Emphasis was placed on the full utilization of the machine s versatility with special attention paid to such parameters as electron energy field size differential attenuation with wax and plaster alignment casts and molds This was the first application of high energy electrons to patients

In 1953 an Allis-Chalmers electron beam donut with magnetic shunt and other associated apparatus was assembled at the Memorial Center in New York

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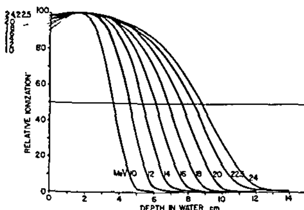


Fig 1 Central axis depth dose data electron beam field size 6 cm × 8 cm

in preparation for the inception of electron beam therapy LAUGHLIN et coll used this technique for extracting high energy electrons from the Memorial betatron, thus making this the second institution to use electrons clinically Although there are many betatrons in use today, the Memorial Center to date probably has the largest series of electron beam patients in the world

The energy was originally determined by adjusting the strength of the magnetic field and expanding the electrons always at the time of maximum field strength However, in the newer model machines, the strength of the magnetic field is not altered, it is always run to maximum The energy of the electrons is determined by the time at which the electrons are suddenly expanded out of the equilibrium orbit This modification was done as it was felt a more mono energetic beam of electrons could be obtained The energy spread in the original technique was approximately 0 1 MeV

The roentgen ray electrons are extracted in the first quarter cycle and after expansion exit on the right of the machine after hitting a platinum pin target The electron beam is expanded in the third quarter and extracted through the left side of the machine where there is no target, i e the electrons are travelling in the opposite direction The electrons subsequently escape through a thin window in the donut in a rectangular configuration Scattering foils are used to disperse the electrons sufficiently to produce a uniform field The foils are made of high atomic number material (such as lead) so as to achieve maximum elec tron deflection with low roentgen ray contamination

The electron beams are monitored by a parallel plate ionization chamber This chamber consists of three dural plates supported in a lucite framework with a 2 mm plate separation The collection voltage is applied to the two outside plates and the center plate is connected to an amplifier In the electron mode

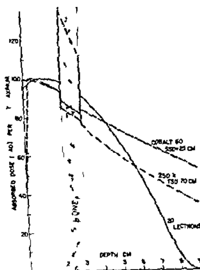


Fig 2 Central axis depth dose data for 250 kV cobalt and 20 MeV electrons corrected for inhomogeneity (bone) field size 10 cm × 10 cm

one obtains about 300 R per minute at a meter on the Allis-Chalmers betatron

Both the energy and the output of the electron beams are calibrated The energy of the electron beam is calibrated using the electro-disintegration threshold of ^{63}Cu which is 10 9 MeV and ^{12}C which is 18 7 MeV The copper and carbon foils are exposed to the electron beam The resultant positron emitting isotopes are counted with a scaler and these two points plotted on the linear calibration curve of the expander circuit By interpolation and extrapolation various energies can be determined

The output is calibrated using a 25 R Victoreen chamber placed in a polystyrene block at a depth of 1 5 cm Using the cobalt 60 correction factor the machine is calibrated for the number of R per count at 20 MeV for a 10 cm × × 10 cm field This is then related to the absorbed dose in rad in patients by the use of radiation chemical dosimeters or by calorimetric methods

One and a half inch lucite is used to collimate the electron beam so as to minimize the production of scatter The cones are constructed in such a manner as to allow the use of inserts for special field shaping the ends of these cones are beveled in order to permit close contact with the skin surface For areas where it is difficult to get good contact with the skin surface due to the thickness of the lucite cones one or more of the walls can be replaced by 1 cm thick aluminum

Depth dose measurements are obtained with air cavity ionization chambers

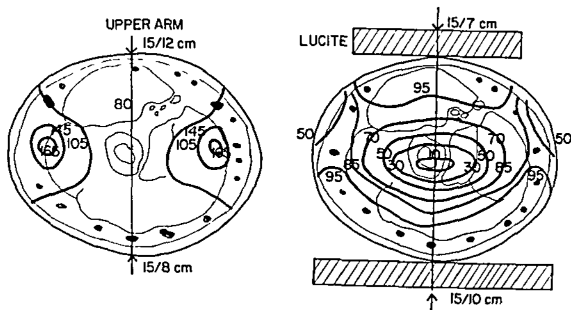


Fig 3 Dose distribution obtained from opposed fields without and with lucite plates 10 MeV betatron electron beam Hodgkin s disease

or P-N junction diodes immersed in a water phantom Central axis dose measurements were made for energies from 10 MeV to 24 MeV with the Allis-Chalmers betatron, and for a variety of circular, rectangular, and square fields The measurement of dose distributions is accomplished by attaching the same kind of air cavity chamber and water pantom to an automatic isodose recorder which automatically plots the dosage points from the various chamber positions Dose distributions were made for both beam axes, parallel to the donut and perpendicular to the donut The greatest variation in the resulting distribution is found in the plan parallel to the donut and for high energies This variation is caused by the geometry of the magnetic shunt The maximum variation in depth dose with field area from 50 cm to 225 cm using these techniques is only 5 %

High energy electrons are not used for their skin sparing effect as are high energy roentgen rays Although the dose to the skin varies with field size and energy, the maximum skin sparing is approximately 10 % The central axis depth doses for a field 6 cm × 8 cm for energies from 10 to 24 MeV are given in Fig 1 As may be seen from the diagram, the main advantage of high energy electron beam therapy is the uniform dose it produces in the first few centimeters of tissue, and the subsequent very sharp fall off of that dose The amount of bremsstrahlung contamination in these energy ranges is between 2 to 4 % If one were to increase the energy much above 35 MeV, the main advantage of

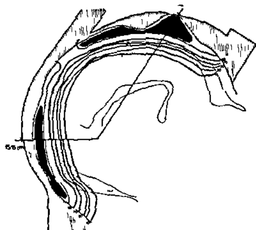


Fig 4 Dose distribution for subcutaneous metastasis to skull 10 4 MeV betatron electron beam

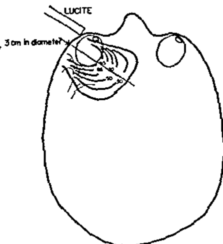


Fig 5 Dose distribution for choroidal metastasis 10 MeV betatron electron beam

high energy electron therapy would be lost because the increase in scattering at these higher energies causes the slope of the depth dose to become much less steep

There is no preferential bone absorption per gram of tissue in these energy ranges This makes it possible easily to correct the corresponding dose distribution for the increased density effect A useful criterion has been found to allow 2 cm unit density absorption to 1 cm bone absorption In Fig 2 are presented the absorbed doses (rad) produced by the absorption of a conventional 250 kV (3 0 mm Cu HVL) roentgen ray beam a cobalt 60 gamma ray beam, and the 20 MeV betatron electron beam for a heterogeneous medium water and bone

In view of such phenomena as sharp fall off and differential increase in depth dose with energy the tumor depths treated with electrons in these energy ranges are limited One would not treat a patient with a single field if the tumor depth were greater than 7 cm nor would one treat a patient with opposed fields if the diameter were greater than 17 cm

A dose distribution from a high energy electron beam is unique in that it does not fall off according to the inverse square law The relative lack of divergence of the beam which is caused by the scattering foils creating a uniform field is of great advantage in treatment planning In patients where the contour is rapidly changing such as post surgical defects, there is no correction for the inverse

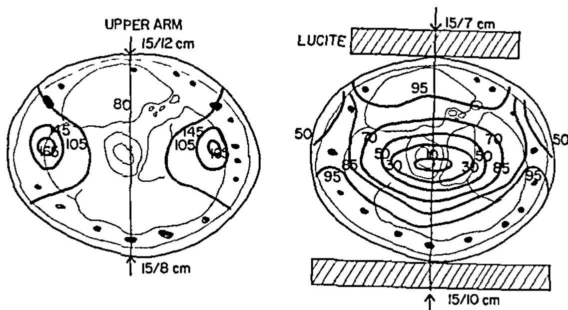


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High energy electrons are not used for their 'skin sparing' effect as are high energy roentgen rays Although the dose to the skin varies with field size and energy, the *maximum skin sparing is approximately 10 %* *The central axis depth* doses for a field 6 cm × 8 cm for energies from 10 to 24 MeV are given in Fig 1 As may be seen from the diagram, the main advantage of high energy electron beam therapy is the uniform dose it produces in the first few centimeters of tissue, and the subsequent very sharp fall off of that dose The amount of bremsstrahlung contamination in these energy ranges is between 2 to 4 % If one were to increase the energy much above 35 MeV, the main advantage of

Table

Comparative values in treatment planning

	Supervoltage cobalt 60	Orthovoltage 250 kV	High energy electrons
1 Depth dose	Non uniform	Non uniform	Uniform
2 Preferential absorption in bone	None/g	Yes	None/g
3 Fall off ratio (tumor/normal)	Poor	Good	Excellent
4 Film dos metry	Good-excellent	Poor	Excellent
5 Ease of field wedging	Difficult	Difficult	Very easy

with a pinhole for the patient to look through while the opposite eye is being treated insures proper rotation of the eyeball

It has long been the opinion of many people in the field of radiologic physics that the drawback of lack of skin sparing could be overcome by using grids To date very little work has been done on it outside of the work of Jacque Ovadia at Michael Reese Hospital in Chicago The phenomenon is that due to scatter below the surface one attains a uniform dose although the center of the field has been blocked out Therefore by making a series of open and closed areas adjacent to one another one can, in effect, obtain a quasi skin sparing effect On this premise a treatment cone jig has been designed by which one may use any combination of open and closed areas to fit the isodose to literally any patient in any circumstance undergoing radiation therapy

In summary the betatron in the electron mode is a highly useful tool in the treatment of patients undergoing radiation therapy where one is able to use single fields where tumor is located at a depth not exceeding 7 cm or where the diameter of the patient in the treatment area is not greater than 17 cm The comparative values that have to be considered in the treatment planning for patients may be judged from the data presented in the accompanying Table

Acknowledgements

The author wishes to thank Dr John S Laughlin of Memorial Hospital New York, for his invaluable help and direction over the years, where most of this work was originally done He also wishes to thank Barbara Edgar for her assistance with the figures

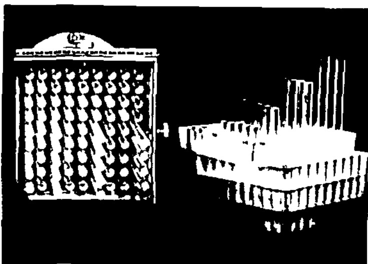


Fig 6 Lucite inserts for treatment cone showing various plates and rods for shaping resultant isodoses

square law These assumptions have been checked using film dosimetry and appear to be quite accurate

The use of polystyrene, lucite or tissue equivalent wax, has been found extremely useful for shaping the electron beam Most head and neck patients treated by the betatron have plaster alignment casts made and, if the initial treatment plan shows the existence of a hot spot, a wax wedge can easily be formed on the cast

The betatron in these energy ranges is particularly useful in the treatment of such areas as breast, nasopharynx, substernal mass, and mammary lymphatic drainage points Also for areas where one desires to avoid such structures as the spinal cord, and yet deliver a uniform dose to cervical nodes

The use of lucite plates to increase the field separation in opposing fields is a convenient method of obtaining a low midplane dose and a high peripheral dose Fig 3 shows an upper arm distribution with and without lucite plates

A difficult problem in treatment planning pertains to subcutaneous metastases to the skull The combination of two oblique fields and wax absorbers with a designed shape to attenuate the beam differentially can produce a uniform subcutaneous dose with minimal brain dose, as seen in Fig 4

Another problem case in the treatment plan is choroidal metastases, and betatron electrons offer an ideal solution One method is exemplified in Fig 5 By placing a lucite wedge in the cone, it is possible to avoid treating the lens A cast

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PROGNOSIS IN HODGKIN'S DISEASE WITH SPECIAL REFERENCE TO HISTOLOGIC TYPE

Results of treatment predominantly by cytostatics

by

A P Andersen H Brincker and F Lass

Hodgkin s disease has been considered by some authors to be of inflammatory or immunologic origin but today it is generally accepted by clinicians as well as by pathologists that the disease belongs to the group of malignant lymphomas It has been widely considered a multifocal systemic disease because of the systemic symptoms but more recent investigations (Rosenberg & Kaplan 1966 Kaplan 1966) have indicated that the disease can just as well be considered of unifocal origin spreading by continuity

The results obtained by intensive radiotherapy in various American and British clinics and presented at the Symposium in Rye New York, in 1966 (Cancer Res vol 26 pp 1044—1311) seem to accord with the unifocal theory and thereby with curability The material now presented is therapeutically charac

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SUMMARY

A brief review of the historical development of the use of high energy electrons is presented Various examples of treatment plans showing the versatility of this modality are given A table showing the comparative advantages and disadvantages of this method of treatment versus cobalt 60 and 250 kV is also presented

ZUSAMMENFASSUNG

Eine kurze Übersicht uber die historische Entwicklung der Verwendung hochenergetischer Elektronen wird gegeben Verschiedene Beispiele fur Behandlungs Plane die die Vielseitigkeit dieser Modalitat zeigen werden angefuhrt Eine Tabelle die die Vorteile und Nachteile dieser Methode vergleichend mit der Cobolt 60 und 250 kV Therapie zeigt, wird ebenfalls gebeben

RÉSUMÉ

L auteur fait un bref rappel de l historique de l emploi des électrons de haute énergie Il donne divers examples de plans de traitement de façon à montrer la souplesse de cette méthode de traitement Il presente aussi un tablau les avantages et les inconvénients de cette methode de traitement par rapport au cobalt 60 et à la roentgen therapie de 250 kV

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Clinical staging was made according to the following three groups

Stage I Disease restricted to one lymphatic region or to two adjacent lymphatic regions on the same side of the diaphragm

Stage II Disease involving more than two lymphatic regions or two lymphatic non adjacent regions on the same side of the diaphgram

Stage III Disease involving the lymphatic system on both sides of the diaphragm or other organs

All stages were also subdivided into clinical types A or B indicating the absence or presence of systemic symptoms i e fever pruritus or an abnormal tendency to sweating

The staging thus differs somewhat from that of PETERS (1950), and PETERS & MIDDLEMISS (1958) as well as that of WESTLING (1965) The classification in four stages (ROSENBERG 1966) which by now is internationally recognized could not be u ed inter alia because only a few of the patients had undergone lymphangiography However stages I and II are identical with the corresponding stages of the named classification

Histologic classification The histologic classification uggested by the Nomenclature Committee (LUKES et coll 1966) was used in the review of the preparations lymphocytic predominance nodular sclerosis mixed cellularity and lymphocytic depletion

A group of ten cases of possible Hodgkin s disease had also to be included Diagnostic Reed Sternberg cells could not be demonstrated on review of these cases but in all other respects the histologic appearances were extremely suggestive of Hodgkin s disease and the cases could not be classified as non specific reactions or as malignant lymphomas of a different nature

Remissions Remission after a given treatment is taken to mean objective improvement with sub idence of pathologic lesions or subsidence of systemic symptoms with or without disappearance of the lesions No distinction could be made between partial and total remissions.

Results

Age and sex distribution The distribution of the material by age and sex is given in Fig 1 In both sexes there is, as in other materials, a preponderance of patients in the age group 20 to 29 years No regard was however paid to the age

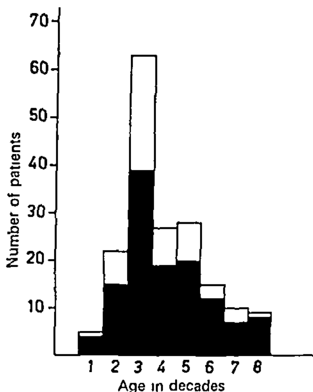


Fig. 1. Distribution of the material according to age and sex: females (55) indicated by white columns and males (124) indicated by black spaces.

terized by previous views holding that Hodgkin's disease is a fatal systemic disease and that the main emphasis must be on chemotherapy. The object of the study has been to report our results and in particular to correlate them to the histologic classifications of the Rye conference.

Material and Method. A total of 195 cases diagnosed as Hodgkin's disease were treated during the period 1st of January 1955 to 31st of December 1964. The histologic preparations in seventeen of these cases could not be procured but in all the others they were reviewed and re-classified by one of the present authors (F. L.) without knowledge of the clinical course.

Ten cases in which the diagnosis could not be maintained were excluded. Of the un-reviewed cases, those in which the histologic report did not mention typical Reed-Sternberg cells were also excluded, i.e. a further six cases. This left 179 cases and in eleven of these the histologic preparations were not reviewed. The material was analyzed as of 31st December 1967, so that the follow-up period was from 3 to 13 years, the follow-up rate being 100 %. A total of 115 cases were treated primarily in the clinic, while 64 were treated secondarily, the primary treatment having been given elsewhere. All survival times have been calculated from the time at which a definite diagnosis was made.

Table 3

Distribution by histologic types compared with the histologic groups of Lukes et coll

Histologic type	Present material*		LUKES BUTLER & HICKS (1966)		
	Number of cases	Per cent	Number of cases	Per cent	Histologic groups
Lymphocytic p edo mina ice	24	17	63	17	L. & H nodular *L. & H diffuse*
Nodular sclerosis	46	32	149	40	Nodular sclerosis
Mixed cellular ty	48	34	97	25	Mixed
Lymphocyt c depletion	24	17	68	18	Diffuse fibrosis Reticular
Total	142	100	377	100	

The groups possible Hodgkin s disease (15 cases) non spec fic changes (at first biopsy) (4 cases) and no revis on (18 cases) excluded

distribution of the general population since a bimodal age specific distribution would then be expected with an accumulation of cases between 20 to 29 years and over 60 (MEIGHAN & RAMSAY 1963)

There were 124 males and 55 females in the material, which gives a male/female ratio of 2 26 1 This is somewhat higher than generally reported i e ROOS & VIDEBAEK (1959) found 1 06 1 MUSSHOFF & BOUTIS (1968) 1 3 1 WESTLING (1965) 1 58 1 while MEIGHAN & RAMSAY (1963) found 2 06 1, and O BRIEN & O BRIEN JR (1954) 3 0 1

Distribution by clinical stage The distribution by clinical stage at the time of diagnosis is presented in Table 1 In 22 cases it was not possible on the basis of the available data to make any clinical staging and in a further 16 cases it was not possible to sub-classify the cases into A or B types While for the material considered as a whole there is a fairly equal distribution within the three clinical stages there seems to be a female preponderance in stage II and a male pre*ponderance in stages I and III* As might be expected there is a marked increase of the systemic symptoms in the more advanced clinical stages

Distribution by histologic type This is presented in Table 2 based upon the revision of the first biopsy specimen ever taken As may be seen nodular sclerosis was more common in females while mixed cellularity occurred more often in males Moreover lymphocytic predominance was found to be extremely rare in women

Table 1

Distribution by clinical stage systemic symptoms and sex

Clinical stage	Females			Males			Total females	Total males	Total females +males
	A	B	?*	A	B	?*			
I	9	1	2	35	4	1	12 (22 %)	40 (32 %)	52 (29 %)
II	17	5	5	11	12	4	27 (49 %)	27 (22 %)	54 (30 %)
III	0	9	0	6	32	4	9 (16 %)	42 (34 %)	51 (29 %)
Unknown			7			15	7 (13 %)	15 (12 %)	22 (12 %)
Total	26 (47 %)	15 (27 %)	14 (26 %)	52 (42 %)	48 (39 %)	24 (19 %)	55 (100 %)	124 (100 %)	179 (100 %)

* Unknown whether systemic symptoms were present

Table 2

Distribution by sex and histologic revision of first biopsy

Histologic type	Females		Males		Total	
	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
Lymphocytic predominance	1	2	23	18	24	13
Nodular sclerosis	20	37	26	21	46	26
Mixed cellularity	17	31	31	25	48	27
Lymphocytic depletion	9	16	15	12	24	13
Possible Hodgkin s disease*	3	5	12	10	15	9
Non specific changes**	1	9	3	14	4	12
No revision***	4		14		18	
Total	55	100	124	100	179	100

* By histologic revision of later biopsy five cases classified according to LUKES et coll (See table 4)

** Histologic revision of later biopsy indicated Hodgkin s disease and classification was made according to LUKES et coll (See table 4)

*** No histologic revision of first biopsy was made but in seven cases a later biopsy was revised and classified according to LUKES et coll (See table 4)

Table 5

Relationship between histologic types and clinical sta e

Histologic types	Number of cases	Per cent of cases in the different clinical stages		
		I	II	III
Lymphocyt c predominance	21	52	29	19 *
Nodular sclerosis	44	36	43	21
M xed cellularity	44	32	34	34
Lymphocyt c depletion	21	19 *	29	5*
Poss ble Hodgkin s disease	11	36**	36*	28*

Th s group contains only 3 cases
* This group conta ns only 4 cases

Relation between clinical stage and histologic type The clinical stage and the histologic type on the first biopsy are compared in Table 5 Only cases in which the clinical stage could be assessed have been included in this comparison It is seen that there is a preponderance of the type lymphocytic predominance in stage I decreasing towards clinical stage III Lymphocytic depletion cor respondingly showed a preponderance of cases in clinical stage III decreasing towards clinical stage I Nodular sclerosis and mixed cellularity the intermediate histologic types and the group of possible Hodgkin s disease exhibit a more even distribution between the three clinical stages

The material of LUKES BUTLER & HICKS had exactly the same tendency as regards the distribution within the clinical stages However these authors re ported a omewhat greater preponderance of the histologic groups lymphocytic or histiocytic proliferation nodular + diffuse within clinical stage I (70 %), but the clinical stages used were not entirely identical The groups diffuse fibrosis and reticular were together most common in stage III (56 %), while nodular sclerosis and mixed cellularity were approximately equally represented in the three clinical stages

Survival in the entire material Fig 2 gives the percentage survival in the entire material a 5 year survival of 33 1 % and a 10 year survival of 13 % These findings agree with those of others in similar series in which radiotherapy and cytostatic treatment has been employed (SHIMKIN et coll 1955 TRUBESTEIN 1956 ROOS & VIDEBAEK 1959 and COOK et coll 1959) However these results do not appear to be up to those obtained by PETERS et coll (1966) by intensive radiotherapy at least not as regards the 10-year survival

Table 4

Evolution of the histologic process in the present series

Primary histologic diagnosis	Secondary histologic diagnosis: Non-specific changes	Possible Hodgkin's disease	Lymphocytic predominance	Nodular sclerosis	Mixed cellularity	Lymphocytic depletion	No revision
Non-specific changes (4 cases)			1			3	
Possible Hodgkin's disease (15 cases)		2	2			3	8
Lymphocytic predominance (24 cases)	2	1	4	1	1	4	11
Nodular sclerosis (46 cases)				10	2	10	24
Mixed cellularity (48 cases)				1	3	21	23
Lymphocytic depletion (24 cases)						13	11
No revision (18 cases)					1	6	11

It is of interest to compare the histologic distribution in the total series with that given by LUKES, BUTLER & HICKS (1966) in their material of 377 patients. They used a histologic classification which comprised six groups of which (1) lymphocytic and/or histiocytic proliferation, nodular, and (2) lymphocytic and/or histiocytic proliferation, diffuse, are identical with the type lymphocytic predominance, while (5) diffuse fibrosis and (6) reticular are identical with lymphocytic depletion. The comparison is apparent from Table 3, in which cases that could not be classified on revision of the first biopsy are excluded, the percentage distribution has been corrected accordingly. The table indicates good agreement as to the distribution in the two materials.

Histologic re-classification on biopsies taken later in the course (33 cases) or on autopsy specimens (58 cases) in a total of 91 cases are listed in Table 4. In only four cases were the changes in the secondary histologic preparations less advanced than in the primary biopsies, while in forty-four the histologic type was more advanced in the secondary specimens. Lymphocytic depletion was present in 54 out of the 58 autopsy specimens reviewed. The findings thus indicate that a change in the histologic appearances may occur in the course of Hodgkin's disease.

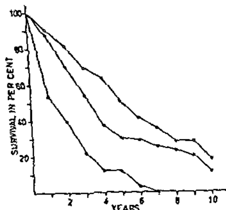


Fig 4 Survival (crude) rate in relation to the clinical stages I (57 patients O—O) II (54 patients ●—●) III (51 patients □—□) (The stage was unknown for 22 patients)

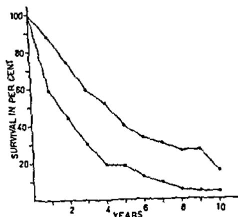


Fig 5 Survival (crude) rate in relation to systemic symptoms no symptoms (78 patients O—O) systemic symptoms (63 patients ●—●) (It was unknown for 38 patients whether systemic symptoms were present)

After using intensive irradiation in the treatment of cases that he called localized lymphadenopathy EASSON (1966) reported a 5 year survival of 55 % and a 10-year survival of 43 % (age corrected survival rates) KAPLAN (1966) in a series treated by megavoltage irradiation gave a 5 year survival of 82 4 % for stages I and II a rate that remained unchanged until the 9th year (actuarial value)

A prognostic comparison of clinical types A and B is presented in Fig 5 which per se of course bear relation to the clinical stage (cf Table 1) Systemic symptoms constitute a serious prognostic sign (cf the above mentioned results of PETERS et coll for stage II A compared with stage II B) LUKES et coll (1966) even recorded poorer 5 year results for stage II B than for stage III A and KELLER et coll (1968) consider systemic symptoms in localized disease to be equally poor prognostically as lymphadenopathy and the dissemination in stage III

Survival in relation to histologic type Table 6 gives the percentage survival within the histologic types and for the group possible Hodgkin s disease at 1 3, and 5 years At 3 years there is a satisfactory correlation with a decreasing number of survivors in the more advanced histologic type At 5 years on the other hand there is a striking fall in the *number* of survivors within the histologic type lymphocytic predominance as compared with the results of LUKES et coll (1966) This phenomenon will be discussed later

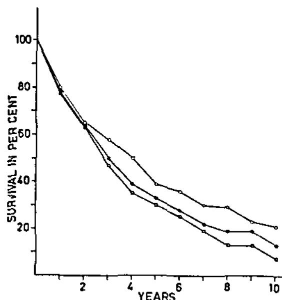


Fig 2 Survival (crude) rate in relation to sex females (55 ○—○) males (124 □—□) entire material (179 patients ●—●)

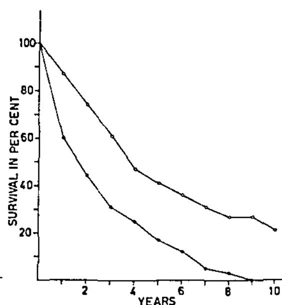


Fig 3 Survival (crude) rate in relation to age under 40 years of age (117 patients ○—○) and over 40 years (62 patients ●—●)

Survival in relation to sex and age Fig 2 also gives the percentage survival for women and men separately Like numerous other workers, the present authors found a somewhat better prognosis in women than in men, 39 % of the female patients living for 5 years as compared with only 30 % of the males further more, 21 % of the female patients lived for 10 years, as compared with 7 % of the males

The prognosis in relation to age is given in Fig 3 The material was divided into two age groups, patients over 40 and under 40 In the older age group the prognosis is distinctly poorer (5 year survival, 17 %) than in the younger (5 year survival, 41 %) This graph does not, however, take into account the higher normal mortality in the older age groups

Survival in relation to clinical stage The percentage survivals in three clinical stages (22 cases excluded because of deficient data) are given in Fig 4 It is evident that the staging places the cases into threee entirely different prognostic groups The results are in keeping with those of WESTLING (1965) but for stages I and II there is a striking difference in relation to materials treated by more intensive irradiation, especially in respect to survivals beyond 5 years Thus, PETERS et coll (1966) reported 5 and 10 year survivals for stage I of 73 % and 52 %, respectively, for stage II A 90 % and 67 % and for stage II B only 23 % and 13 %

Table 6 *(cont)*

		Lukes Butler & Hicks (1966)		
5 years		5 years		
Number at risk	Survival per cent	Number at risk	Survival per cent	Histologic groups
22	27	63	73	L. & H nodular
				L & H diffuse
36	44	149	44	Nodular sclerosis
40	15	97	39	Mixed
17	24	68	13	Diffuse fibrosis
13	69			Reticular

* The groups non specific changes (at first biopsy) and no revision excluded

(tumour dose) but the fields were generally rather small Secondary radiotherapy was given with a purely palliative object, directed at local lesions causing symptoms

Table 7 presents the primary treatment in relation to clinical stage As might be expected irradiation was most often used in clinically localized stages If chemotherapy was used in localized stages this was mainly in cases where the condition was characterized by systemic symptoms However, chemotherapy without concomitant radiotherapy, in these stages is not in keeping with present views concerning the treatment of Hodgkin s disease (Karnofsky et coll 1963 Aisenberg 1964 Karnofsky 1966 Perry et coll 1967)

Chemotherapy From the total of 179 cases 152 received at some stage between one to twelve courses of one or more out of a total of 15 cytostatic substances Chemotherapy alone as the primary treatment (Table 8) was given to 56 while the others had the courses at later stages when the disease was more or less clinically advanced or generalized A total of 468 courses were administered but in some cases the cytostatic medication was combined with irradiation or with steroid agents so it was not possible to assess the cytostatic effect per se Several cases moreover received two cytostatics in combination but those used for this treatment varied so that for numerical reasons the combined treatment with cytostatics cannot be assessed Lastly, several single courses were excluded because data for estimating the length of the remission were absent This leaves 307 single courses that could be evaluated

Table 6

Survival according to histologic types compared with the histologic groups of Lukes et coll

Histologic type	Present material*			
	1 year		3 years	
	Number at risk	Survival per cent	Number at risk	Survival per cent
Lymphocytic predominance	24	83	24	63
Nodular sclerosis	46	93	46	61
Mixed cellularity	48	71	48	38
Lymphocytic depletion	24	54	24	21
Possible Hodgkin s disease	15	93	15	80

Survival in relation to duration of symptoms before diagnosis In Fig 6, the material is divided into three groups by duration of symptoms Sufficient data were however not available in 31 of the cases There does not seem to be any definite difference between the three groups, so that in the present material it is not possible to assess the prognostic significance of the duration of symptoms

Treatment

It is only natural that the treatment varied a good deal seeing that the material comprises a period of ten years A number of cytostatic substances have been launched on the market during this period and used for such a short time on so few cases that it is impossible to draw conclusions Most cases were treated several times by irradiation, cytostatics or steroids, and frequently these types of treatment have been used in varying combinations Numerous factors are accordingly difficult to assess but an attempt will be made to point out a few features

Irradiation About 160 to 250 kV radiation was used during the greater part of the period Telecobalt was employed for only a few cases in the last years of the study period The dosage varied a good deal but with a tendency to higher dosage in recent years Cases of localized disease treated primarily by irradiation received a dosage generally in the range 1 500 to 2 500 R over two to three weeks

Table 6 *(cont.)*

		Lukes, Butler & Hicks (1966)		
5 years		5 years		
Number at risk	Survival per cent	Number at risk	Survival per cent	Histologic groups
22	27	63	73	L. & H. nodular L. & H. diffuse
6	44	149	44	Nodular sclerosis
40	15	97	37	Mixed
17	24	68	13	Diffuse fibrosis
				Reticular
13	69			

* The groups non-specific changes (at first biopsy) and no revision excluded

(tumour dose) but the fields were generally rather small. Secondary radiotherapy was given with a purely palliative object, directed at local lesions causing symptoms.

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Chemotherapy. From the total of 179 cases, 152 received at some stage between one to twelve courses of one or more out of a total of 15 cytostatic substances. Chemotherapy alone as the primary treatment (Table 8) was given to 56, while the others had the courses at later stages when the disease was more or less clinically advanced or *generalized*. A total of 468 courses were administered, but in some cases the *cytostatic* medication was combined with irradiation or with steroid agents so *it* was not possible to assess the cytostatic effect per se. Several cases moreover *received* two cytostatics in combination, but those used for this treatment varied, *so that* for numerical reasons the combined treatment with cytostatics cannot be assessed. Lastly, several single courses were excluded because data for estimating *the* length of the remission were absent. This leaves 307 single courses that could be evaluated.

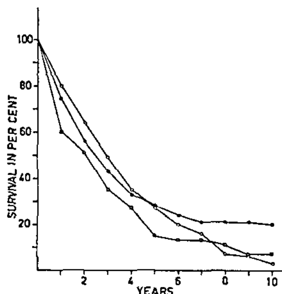


Fig 6 Survival (crude) rate in relation to duration of symptoms 0 to 5 months (88 patients ○—○) 6 to 11 months (37 patients □—□) over 12 months (23 patients ●—●) (Data missing for 31 patients)

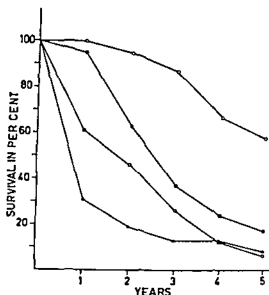


Fig 7 Survival (crude) rate in relation to duration of initial remission no remission (△—△) remission 1 to 6 months (□—□) remission 7 to 12 months (●—●) remission over 12 months (○—○)

The dependence of the prognosis upon the duration of the remission obtained by the primary treatment was analyzed regardless of whether it consisted of irradiation, chemotherapy, or a combination of both

The result is presented in Fig 7 In the cases that obtained no remission at all after the initial treatment, the prognosis appeared to be serious, even at short sight, only 31 % surviving for a year In the group of cases that obtained initial remission of from 1 to 6 months, only 6 % survived for 5 years, while the 5 year survival among the group having an initial remission exceeding 12 months was 58 % The investigation thus indicates that the longer the initial remission the better the prognosis A long initial remission may be a sign of slower progression of the disease, a factor that has been pointed out by Cook et coll (1959) and Karnofsky et coll (1963) Musshoff et coll (1966), on the other hand considered the remission rate and the duration of the first remission to be indications of therapeutic effectiveness

In Table 8 are listed the six most common cytostatics representing 234 single courses without maintenance therapy The remaining 73 courses are distributed over a total of nine additional cytostatics so that these numerical values are too small for comparison The table also gives the average duration of the remissions with the drugs used It will be seen that Natulan induced the longest

Table 7

Primary treatment according to clinical stage

Primary treatment	Clinical stage			Unknown	Total
	I	II	III		
Irradiation	8	28	12	14	[illegible]
Chemotherapy	6	18	29	3	56
Irradiation + chemotherapy	1	5	4	2	12
Miscellaneous types of treatment	7	3	6	3	19

remissions The table also indicates the number of cases in which no remission was obtained More than one course of the same drug was given in a number of cases The duration of the remissions after the second and third courses was always shorter than after the first in all the cases in which more than two courses were available as a basis for the calculations This indicates that secondary cytostatic therapy is less effective than primary therapy a certain form of resistance developing on continued use of the same chemotherapeutic agent

A comparison of the drugs triethylene melamine, cyclophosphamide Natulan and vinblastine is presented in Fig 8 The most striking feature is that Natulan and vinblastine give a larger number of remissions than the alkylating substances triethylene melamine and cyclophosphamide In addition, it may be seen that with Natulan a larger number of long lasting remissions was obtained than with the other drugs On the other hand *no remission lasted beyond* 6 months on vinblastine The alkylating agents triethylene melamine and cyclophosphamide possessed approximately the same effectiveness *With triethylene* melamine especially there were only a few remissions longer than 6 months

Discussion

Definite information concerning the spontaneous course of Hodgkin's disease is lacking as only very few series of untreated cases have been published EWING in an early paper (1940) *reported an average duration* of 18 months SHIMKIN et coll (1955) collecting a total of 433 cases from the literature prior to 1936 reported a 5 year survival of less than 15 % from the time of diagnosis They compared this result with 1 109 cases collected from the period after 1940 among which the 5 year survival was 26 6 % This difference is statistically significant They mentioned *that factors* other than the treatment may have contributed to this difference but avoided drawing further conclusions

Table 8

The six most frequently used drugs number of courses average duration of remission and number with no remission

Drug	One course	Two courses	Three courses	Four courses
Diepoxybutan	14	2	0	0
Triethylene melamine	85	26	7	1
Cyclophosphamide	26	5	1	0
Vinblastine	21	2	0	0
Vincristine	6	3	0	0
Natulan	29	6	0	0

At all events, the prognosis of Hodgkin s disease has improved in recent years, particularly in series where intensive radiotherapy has been used The present results are in keeping with this trend

Various factors influence the prognosis, inter alia the age The prognosis is poorer in the older group of cases (Fig 3), a factor explained by JACKSON & PARKER (1947) as a higher incidence of a sarcomatous histologic appearance, while UDDSTROMER (1934) believed that elderly patients had a greater tendency to generalization of the disease

Sex is undoubtedly another prognostic factor (Fig 2) However, doubts have been raised as to whether the apparently better prognosis in women is real EPSTEIN (1939), PETERS et coll (1966) and WESTLING (1965) found the same sex difference but without a statistical significance and SHIMKIN et coll (1955) reported a significant sex difference only for stage I MEIGHAN & RAMSAY (1963) recorded a better prognosis only in women of fertile age a phenomenon also pointed out by BICHEL (1955) VIDEBAEK (1950) could not demonstrate any sex difference, and MUSSHOFF & BOUTIS (1968) believed that the better prognosis in women was due to a lower incidence of the more advanced clinical stages The better prognosis in women in the present material may have been due to the lower incidence of clinically more advanced stages

The clinical stage at diagnosis is an important prognostic factor (Fig 4) and this seems to be generally accepted Since the clinical stage no doubt bears some relation to the histologic appearance (Table 5), the latter must also be considered of importance, although, unlike LUKES et coll (1966), we failed to notice so benign a course of the histologic type known as lymphocytic predominance Incidentally, it has been stated by HILTON & SUTTON (1962) that Hodg-

Table 8 *(cont)*

Total number of courses	Average duration of remission in months				No remission	
	Total courses	First course	Second course	Third course	Number of courses	Per cent
16	1 4	1 1	(3 5)		8	50 0
119	2 2	2 5	1 5	1 6	41	34 4
32	2 9	3 4	0 8	(2 0)	11	34 4
23	3 0	3 0	(4 0)		3	13 0
9	3 4	4 3	1 7		1	11 1
35	5 0	5 7	2 0		2	5 7

kin s disease is the only group of malignant lymphomas in which clinical staging is of any value from the prognostic point of view

The duration of the disease before diagnosis (Fig 6) has by *some authors also* been related to the prognosis PETERS & MIDDLEMISS (1958) for instance regarded the 5 year survival to be the better the longer the disease had lasted before treatment Later PETERS et coll (1966) noted a considerable preponderance of 10-year survivors with symptoms for more than one year WESTLING (1965) on the other hand reported the best 5 and 10 year survivals in cases with symptoms for only 0 to 6 months but the difference as compared to the other groups was not significant

Finally the duration and the completeness of the remission on initial treatment is obviously of prognostic importance as is also apparent from Fig 7

During the past 50 years several attempts have been made to correlate the histologic appearances in Hodgkin s disease partly to the prognosis and partly to the anatomical spread of the disease JACKSON & PARKER (1944) used a classification into three groups paragranuloma granuloma and Hodgkin s sarcoma The first group represented a benign and slow course the third group a malignant and short course The main objection to this classification was that the group of paragranulomas made up only 7 to 8 % of the cases and the group of Hodgkin s sarcoma only 2 to 3 % while the intermediate group of granulomas represented about 90 % In this group therefore there was a wide histologic and thus also prognostic variation

LUKES et coll (1966) originally used a classification comprising six groups which however was reduced to four types by the Nomenclature Committee We felt that it might be of interest to test this classification by review and re classification of the histologic preparations This revealed conformity as regards the distri-

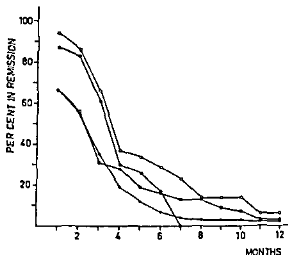


Fig 8 Duration of remission following treatment with different drugs Natulan (35 courses ○—○) vinblastine (23 courses ●—●) cyclophosphamide (32 courses □—□) triethylene melamine (119 courses △—△)

bution of the material within the histologic types (Table 3) and the distribution in relation to clinical stage (Table 5), with due consideration of the fact that our material was less than half as large as that submitted by LUKES et coll The main disagreement was, as already mentioned, the 5 year survival in the group 'lymphocytic predominance' (Table 6) We cannot explain this disagreement As emphasized by KELLER et coll (1968) one of the difficulties with Lukes' classification is to distinguish between the types mixed cellularity and lymphocytic predominance We may have placed some of the former group into the latter, and this would account for the discrepancy This does not seem particularly likely, however, as the type lymphocytic predominance' in our material makes up exactly the same proportion (17 %) of the total series as in that of LUKES et coll On the other hand KELLER et coll found this proportion to be only 5 % Another contributory cause of the poor prognosis with this histologic type in the present series may be that while only 52 % of the cases in this group were in clinical stage I, LUKES et coll had 70 % in this stage Furthermore, there was only one woman in the group, so that it is predominated entirely by the prognostically more unfavourable male patients The favourable prognosis in lymphocytic predominance may be due to immunologic defence mechanisms, suppressed by the extensive use of chemotherapy so that the 5 year survival is poorer than that obtained by others who have used irradiation to a greater extent This is of course mere hypothesis which cannot even be supported by the present findings The histologic type nodular sclerosis has been emphasized by some workers (PERRY et coll 1967) as the most difficult group to classify according to Lukes system Nevertheless we found conformity with the material of LUKES et coll for this group as well as in respect to the 5 year survival

We found that in a large number of the cases in which two biopsies had been performed in the course of the disease the histologic appearances had altered (see Table 4) This alteration was in a more malignant direction in the majority of the cases However it is well known that different histologic Hodgkin changes within different groups of lymph nodes may be present in the same subject Such differences may even occur within the same group of nodes or in the same histologic section (CUSTER & BERNHARD 1948 RUTTNER 1953) This may be the explanation of the change towards a more benign appearance that was noted in a few cases a phenomenon also reported by CUSTER & BERNHARD However the predominant number of cases with changes in the histologic appearances undergo a transformation in a more malignant direction It may thus be expected that in a certain proportion of cases at least the clinical and histologic progression is concurrent Histologic progression has also been demonstrated by CUSTER & BERNHARD (1948) JELLIFFE & THOMSON (1955) TRUBESTEIN (1956), and WESTLING (1965) It may be that other factors e g bacterial viral or fungal infections that often affect these patients in the terminal stages (CASAZZA et coll 1966) may also be contributory The same applies to a possible effect of irradiation and chemotherapy However CUSTER & BERNHARD failed to demonstrate that irradiation alone could induce transformations in the histologic process either into a more malignant or a more benign direction (presupposing that the lesion had not healed during the treatment) Let it be emphasized that among the twenty two cases of nodular sclerosis in which new biopsies were performed later in their course twelve presented evidence of conversion to more malignant histologic types viz two to mixed cellularity and ten to lymphocytic depletion LUKES et coll (1966) were unable to demonstrate with certainty any histologic evolution in nodular sclerosis but felt that it presumably existed

It would appear that according to the view prevailing to-day isolated chemotherapy is not the treatment of choice for Hodgkin s disease in localized clinical stages I and II Intensive radiotherapy is indicated in such cases We must admit that the treatment of our material does not accord with these principles However chemotherapy still remains of value for certain groups of cases in Hodgkin s disease Although it is accepted that clinically less advanced stages of Hodgkin s disease may be cured by irradiation a number of these cases will later become generalized and a not inconsiderable proportion have primarily been disseminated In such instances chemotherapy is still of great value

Chemotherapy may be administered in various ways with or without irradiation as courses of one drug with or without maintenance therapy or as combination of two or more drugs

The results obtained by chemotherapeutic management in our series (Table 8 and Fig 8) cannot be compared directly with the results of others In part

our results are based upon a retrospective study, not upon a controlled clinical series, and in part the definition of remission may vary Within our own series, however, the various drugs may be compared, but for numerical reasons a relative assessment is permitted only for the individual drugs, not for combined medication

Three factors must be stressed in evaluating the effectiveness of a cytostatic In the first place the ability to induce remission and in the second place the duration of the induced remission must be considered The third factor is the influence upon the patient s survival

There is little doubt, on the basis of such a comparative evaluation of the cytostatics in the present series, that Natulan was the most effective, both in respect to the duration and the frequency of remissions (Table 8) Moreover, Natulan induced a relatively larger number of long lasting remissions, i e remissions sustained for up to one year (Fig 8) Other authors (MATHE et coll 1963, DEVITA et coll 1966, SAMUEL et coll 1967) have also experienced encouraging results in clinical experiments with Natulan in Hodgkin s disease Vinblastine and vincristine produced only a slight increase in the average duration of remissions, compared with the alkylating substances, but there was a marked increase in the incidence of remissions Long lasting remissions were not common with vinblastine (Fig 8)

As may be seen from Table 8, when the same drug was used there was a decrease in the average duration of remission in courses administered after the primary one and, as has been demonstrated repeatedly, resistance develops in the event of continued treatment with the same substance or group of substances However, this need not entail cross resistance to other groups, which is often a difficult problem to assess, the secondary treatment usually being administered at a time when the disease is more advanced clinically as well as histologically and when the patient s general condition is poorer partly because of the disease and partly because of previous treatment which may have compromised the normal biologic defence mechanisms

It is difficult to say whether cytostatic treatment, administered alone, prolongs survival in Hodgkin s disease, and the present material does not allow of any conclusions SHIMKIN et coll (1955) failed to demonstrate that HN (nitrogen mustard) was able to prolong life Others (ROOS & VIDEBAEK 1959, KARNOFSKY et coll 1963 KARNOFSKY 1966) felt that life may be extended secondarily by improving the general condition through cytostatic therapy However it must be borne in mind that during the very period that cytostatics have been used a number of other therapeutic measures, such as modern antibiotics and corticosteroids, have been introduced and these too may constitute contributory aids PERRY et coll (1967) have expressed the most optimistic view in relation

to this problem stating that proper manipulation of the available chemotherapeutic substances may produce such destruction of the malignant cells that this per se prolongs the life of the patient

Conclusions

A 10-year material (1955—1964) comprising 179 cases of Hodgkin's disease is reported Chemotherapy was used to a great extent but irradiation was employed as well, mainly for palliative purposes The total 5 year survival rate was 33 1 % and the 10 year survival rate 13 % The poorest prognosis was in (1) cases in an advanced clinical stage (2) cases with systemic symptoms (3) elderly patients (4) male patients and (5) patients with short and incomplete remission after the primary treatment The duration of symptoms before the diagnosis did not appear to play any prognostic role

All histologic preparations but eleven were reviewed and re classification was made into the histologic types suggested by the Nomenclature Committee (LUKES et coll 1966) There was agreement with the material of LUKES BUTLER & HICKS (1966) in respect to distribution within the histologic type, relation to clinical stage and prognosis within the histologic types save the lymphocytic predominance type in which the prognosis was considerably poorer than reported by LUKES et coll This finding is discussed in more detail Signs of evolution in a more malignant direction of the histologic types with clinically progressing disease also within the type nodular sclerosis were apparent

Natulan was the most effective of the various cytostatic drugs both in respect to the length of remission and the ability to induce remission

Acknowledgements

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SUMMARY

A total of 179 cases of Hodgkin's disease treated predominantly by chemotherapy during the period 1955—1964 were reviewed histologically and reclassified into the histologic types recommended by the Nomenclature Committee (LUKES et coll 1966) The 5 year survival rate was 33 1 per cent Natulan® proved to be the most effective of the cytostatic drugs

ZUSAMMENFASSUNG

In 179 Fällen der Hodgkin'schen Erkrankung die hauptsächlich mit Chemotherapie während der Periode 1955—1964 behandelt wurden wurden die Befunde histologisch überprüft und in den von der Nomenclature Committee (LUKES et coll 1966) empfohlenen histologischen Typen reklassifiziert Die 5-Jahre Überlebenszeit war 33 1 % Natulan® erwies sich als das beste der cytostatischen Mittel

RÉSUMÉ

Les auteurs ont revu histologiquement et reclassifie d apres les types histologiques recom mandés par le Comité de Nomenclature (LUKES et coll 1966) un total de 179 cas de maladie de Hodgkin traites principalement par chemothérapie au cours de la periode 1955—1964 Le taux de survie a 5 ans etait de 33 1 pour cent La Natulan® etait le plus efficace des medicaments cytostatiques

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FROM THE DEPARTMENT OF GYNECOLOGIC RADIOTHERAPY (DIRECTOR OLLE KJELLGREN), UNIVERSITY OF UMEÅ, SWEDEN

EFFICACY OF PRIMARY RADIATION IN CARCINOMA OF THE ENDOMETRIUM

Preliminary study of unselected material from a specific region in the north of Sweden

by

O KJELLGREN and S S MAGNUSSON

Carcinoma of the corpus uteri is notifiable in Sweden Analysis of the Cancer Registry records (see ref 27) reveals that endometrial carcinoma constitutes 6 % of all malignant neoplasms in women The annual incidence rate is 17 per 100 000 women, and 600 to 650 new cases are reported every year The probability that a Swedish woman will develop endometrial carcinoma sometime during her lifetime is 1 in 66 It is a disease of later life and is rare in women under the age of forty (1 %) In women over forty years of age, the incidence rate is 37 per 100 000 women Between fifty nine and seventy nine years of age, the incidence rate is about 47 per 100 000 women, reaching a peak of 57 per 100 000 women in the 65 to 69 year group (Fig 1) The age distribution in the 2 524 cases reported in Sweden during the period 1958—1961 is given in Fig 2

The management of carcinoma of the endometrium has for years been a subject of controversy all over the world Two main lines of treatment are advocated primary surgery with or without postoperative radiation, and primary

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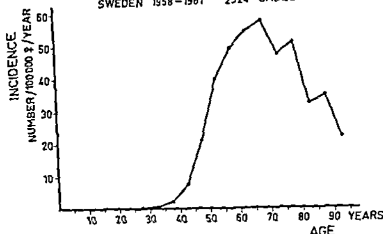


Fig 1 Incidence of endometrial carcinoma in different age groups in Sweden

radiation with or without supplementary operation (2—10 12—17 20—23 29—30) Most authorities now agree that primary surgery for early adenocarcinoma of the highly differentiated type gives excellent results it should be followed by vaginal vault irradiation in order to decrease the risk of vaginal recurrence This procedure reduces the incidence of recurrence from 12 to 3 or 4 % (3 16 24 28) In late cases with advanced changes management is more difficult These growths even if highly differentiated should probably be irradiated prior to operation In carcinoma of the undifferentiated type primary radiation followed by total hysterectomy and bilateral salpingo-oophorectomy is the treatment of choice The prognosis in anaplastic carcinoma treated by primary surgical resection is very poor even if the tumor is localized to the uterine cavity (17)

Evaluation of the clinical results of treatment of endometrial carcinoma is beset by many difficulties Although a vast literature has grown up around the subject the diversity of methods of management has made it difficult to collect a significant number of cases treated by any single technique Another difficulty is the matter of classification As KOTTMEIER (16) has pointed out there is no justification in comparing statistics based on surgically classified cases with those collected from clinically classified cases. Finally and most importantly the problem of patient selection arises If the significance of this is disregarded or

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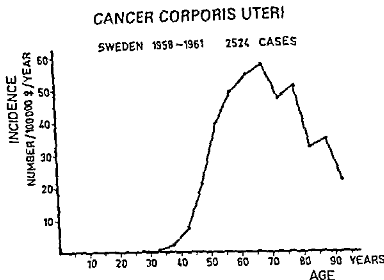


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Evaluation of the clinical results of treatment of endometrial carcinoma is beset by many difficulties. Although a vast literature has grown up around the subject the diversity of methods of management has made it difficult to collect a significant number of cases treated by any single technique Another difficulty is the matter of classification As KOTTMEIER (16) has pointed out, there is no justification in comparing statistics based on surgically classified cases with those collected from clinically classified cases Finally and most importantly the problem of patient selection arises If the significance of this is disregarded or

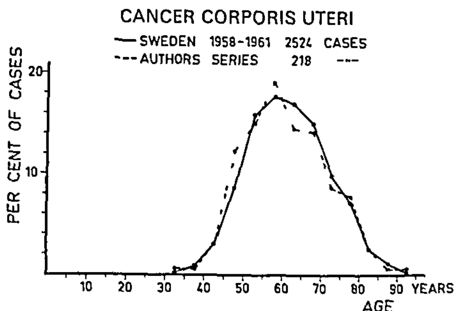


Fig. 2. Age distribution in the material compared to that of a larger Swedish series.

minimized, unwarranted conclusions can too easily be drawn. For example, the results obtained with primary surgical resection followed by intravaginal radium are statistically excellent. It is, however, often overlooked that the case material on which these results are based is always highly selected, since it does not include cases of tumors that are either advanced or disseminated, nor those in which operation is contraindicated by concomitant disease. Furthermore, the criteria by which the cases are selected are often obscure, if given at all.

Many of the protagonists of primary surgery maintain that radiation is an ineffective form of treatment for endometrial carcinoma, pointing out that the 5 year recovery rates are much inferior to those obtained with primary surgery. A moment's reflection will reveal the fallacy in this reasoning. In most countries, the cases referred to radiotherapy centres are the unfavourable ones, those that have not been accepted for primary operation for the reasons stated above. In other words, the element of selection is again just as much in evidence but this time the factors are weighted the other way. It should be stressed that the excellent results of primary radiation at Radiumhemmet have been achieved in a selected material, since about 20 % of the cases referred to the institution have already been operated upon elsewhere. Incidentally, KOTTMEIER has never advocated radiation alone as the treatment of choice for endometrial carcinoma on the basis of these results, as has sometimes erroneously been maintained, but he advocates that operation should always be preceded by radiation.

With reservation for the obviously limited value of statistics, where the treatment of endometrial carcinoma is concerned the following figures give an idea of what can be achieved with different methods of management (19) Primary surgery followed by intravaginal radium, in selected cases of early malignancy confined to the endometrium or invading the inner half of the myometrium gives a 90 % 5 year recovery rate In more advanced cases with invasion of the outer half of the myometrium it is about 60 % and in cases of pelvic recurrence treated with radiotherapy after primary surgery it is 40 % The 5 year recovery rate for cases in stage I group 1 (clinically operable) treated with primary intracavitary radium with the Heyman packing technique alone is 85 %, the corresponding figure for stage I, group 2 (technically operable) cases is about 50 % Cases which have been secondarily operated upon because of a recurrent growth have been included in these figures, the incidence of such operations is about 14 % The recovery rate for cases operated upon because of failure of radiotherapy is about 60 % (ref 12—16)

GRAHAM writing in 1956 on the treatment of choice for carcinoma of the endometrium stated that he knew of no papers in which all the cases from a given geographic area were included We are now able to present the preliminary results of such a study Our department which has full radiologic and surgical facilities serves a specific region in the northern part of Sweden with about 700 000 inhabitants There are no other radiotherapy centres in the area All cases of possible or manifest endometrial carcinoma in this region are referred to the department for review and treatment By arrangement with the gynecologic and surgical services within the area no therapeutic procedures are carried out on these cases prior to their admission to our department The only exceptions are occasional cases in which endometrial carcinoma has been an incidental finding at hysterectomy for some other condition and which are thereafter referred to the department for postoperative radiotherapy This therapeutic centralization has made it possible to carry out a uniform plan of management on an unselected material comprising all the known cases of endometrial carcinoma occurring in a specific region The preliminary results of a therapeutic trial of this nature are presented in this paper The efficacy of primary radiation for stage I and stage II endometrial carcinoma determined by the microscopic study of specimens obtained at major surgery or post radiation curettage is discussed The term *primary cure rate* conveniently expresses the proportion of cases in which no residual malignancy was evident

Material Between 1963 and 1967, 242 cases of malignant tumors of the corpus uteri were treated A total of 218 cases (90 %) had endometrial carcinomas and these make up the material The remaining 24 cases (10 %) of

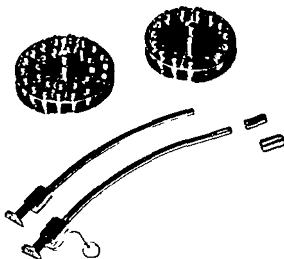


Fig 3 Instrumentarium used for intrauterine packing Two jacket holders are seen uppermost two applicators of which the lower one is loaded with an irradiator with thread and tag are seen below and to the right are two jackets of different size

arcoma have been excluded from the study The age distributions, in our material and in the 2 524 cases registered at the Swedish Cancer Registry during the period 1958—1961, are given in Fig 2 The material is unselected and is made up of all known cases of endometrial carcinoma occurring over a period of three years in the region served by the department The staging of invasive carcinoma of the corpus uteri according to the international classification is as follows

Stage I The carcinoma *is confined to the corpus*,

Stage II The carcinoma has involved the corpus and the cervix,

Stage III The carcinoma has extended outside the uterus but not outside the true pelvis

Stage IV The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum

According to this classification our material included 158 cases (75 6 %) in stage I, 32 cases (15 8 %) in stage II, 7 cases (3 3 %) in stage III, and 11 cases (5 3 %) in stage IV

Management Since 1963 the department has carried out a clinical experiment whereby all cases of endometrial carcinoma irrespective of the clinical stage, have been treated with primary radiation Intracavitary and vaginal radium was the initial treatment selected in most cases (200) and external radiotherapy in the remainder

Evaluation of the patient s general state of health was made on admission and the presence of associated conditions such as obesity, hypertension, diabetes or cardiovascular disease was noted This was followed by a pelvic examination

Table I

Irradiators used in treatment of carcinoma of the uterine corpus

ngth m	Diameter mm Inner	Outer	Wall thickness mm steel	Radium tube	Total filtration mm Pb	Dose rate at 1 5 cm
ma ntly sealed irrad tor (No 0)						
0	3	6	1 5	Radium content 10 mg External length 17 mm Active » 10 mm Diameter 2 9 mm Filtration 2 mm Pb	2 4	32 2 rad/h = 100 %
Add t on l jackets						
2 5	6 1	8	1 No 1		2 6	30 8 rad/h = 96
23 5	6 1	10	2 No 2		2 7	29 9 rad/h = 93

under anesthesia and cervical dilatation and fractional curettage in order to determine uterine size and the depth and extent of tumor involvement The procedure was carried out by a senior staff member with both gynecologic and radiotherapeutic qualifications After clinical staging a preliminary plan of management was drawn up Treatment was usually instituted forthwith as most of the patients had previously undergone curettage at local hospitals In a few cases the uterine neoplasm was sometimes found to be secondary to carcinoma of the cervix or the ovaries and these cases have of course been excluded

The cases in stage I and stage II were treated with intracavitary radium The Heyman packing methods as modified by KJELLGREN & JONSSON (11) was used in 187 cases (98 4 %) The uterus was packed on three separate occasions fortnightly it was sometimes necessary to insert a tandem at the third application on account of uterine shrinkage The uterus was so atrophic in three cases 1 6 %) that adequate packing could not be carried out and a tandem was inserted on each occasion

Capsules containing 10 mg of radium were used for packing The details of size and filtration are given in Fig 3 and Table 1 The number and size of the capsules determined the duration of irradiation in each individual case (Table 2) The estimated delivered radiation dose at a distance of 1 5 cm from the nearest radium source was 3 000 rad

The packing technique is simple The idea is to fill the cavity with radium and if possible to distend and thin out the walls thus shortening the distance

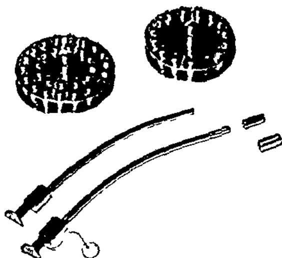


Fig 3 Instrumentarium used for intrauterine packing Two jacket holders are seen uppermost two applicators of which the lower one is loaded with an irradiator with thread and tag are seen below and to the right are two jackets of different size

sarcoma have been excluded from the study The age distributions, in our material and in the 2 524 cases registered at the Swedish Cancer Registry during the period 1958—1961, are given in Fig 2 The material is unselected and is made up of all known cases of endometrial carcinoma occurring over a period of three years in the region served by the department The staging of invasive carcinoma of the corpus uteri according to the international classification is as *follows*

Stage I The carcinoma is confined to the corpus,

Stage II The carcinoma has involved the corpus and the cervix

Stage III The carcinoma has extended outside the uterus but not outside the true pelvis,

Stage *IV The carcinoma has extended outside the true pelvis or has obviously* involved the mucosa of the bladder or rectum

According to this classification our material included 158 cases (75 6 %) in stage I, 32 cases (15 8 %) in stage II 7 cases (3 3 %) in stage III, and 11 cases (5 3 %) in stage IV

Management Since 1963 the department has carried out a clinical experiment whereby all cases of endometrial carcinoma irrespective of the clinical stage have been treated with primary radiation Intracavitary and vaginal radium was the initial treatment selected in most cases *(200)* and *external radiotherapy in the* remainder

Evaluation of the patient s general state of health was made on admission and the presence of associated conditions such as obesity hypertension, diabetes or *cardiovascular disease was noted* This was followed by a pelvic examination

Table 3

Type of treatment in carcinoma of the uterine corpus stage I to II

	Cases treated	
	Number	Per cent
Intracavitary radium eventually followed by external radiation	200	91 7
External radiation only	8	3 7
Primary surgery	10	4 6
Total	218	100

Table 4

Method of judging the effect of primary radiotherapy in carcinoma of the uterine corpus stage I and stage II

	Number of cases		Total	Per cent
	Stage I	Stage II		
Total hysterectomy ± bilateral salpingo-oophorectomy	99	20	119	63
Control curettage	59	11	70	37
Total	158	31*	189	100

One additional patient died of intercurrent disease one week before control curettage was planned

Of the 208 cases in all stages treated with primary radiotherapy 200 received intracavitary radium (Table 3)

Six weeks after the completion of the radium treatment, total hysterectomy and bilateral salpingo-oophorectomy was performed in all suitable cases with stage I or stage II carcinoma In the poor surgical risk cases where operation was considered inadvisable because of e g old age hypertensive cardiovascular disease or diabetes dilatation and curettage were carried out three months after radiation Table 4)

Primary surgery was performed in only ten cases of the total material (Table 3) These operations were performed at local hospitals as a result of the incorrect interpretation of the clinical or histologic findings and the cases were later referred to the department for further care

Table 2

Treatment times in hours required to deliver 1 000 rad at 1.5 cm depth with different numbers and sizes of irradiators in the treatment of carcinoma of the uterine corpus

Number of irradiators	Irradiator size		
	0	1	2
6	15 1/2	19	21 1/2
7	14 3/4	18	20
8	14	17	18 3/4
9	13	16 1/4	17 1/2
10	12 1/4	15 1/4	16 1/2
11	11 1/2	14 1/2	15 1/2
12	10 3/4	13 3/4	14 3/4
13	10 1/4	13 1/4	14
14	9 3/4	12 3/4	13 1/2
15	9 1/4	12 1/4	13

between the radium and the outermost layers of the uterus. Each capsule has a thread attached, which is tagged and numbered for easy removal. The cavity is packed in rows from the fundus downwards, the length of thread lying outside the vagina and held in the hand, indicating the position of the capsules. The cavity is packed as tightly as possible: to accomplish which Hegar's dilators may be carefully burrowed between the capsules lying in the uterus to make room for more. The normal uterus is not readily distensible, but a uterus which is the site of a carcinoma can usually be ballooned out, thus ensuring satisfactory contact between the radium and the tumor. In experienced hands, perforations are uncommon and, as will be shown later, rarely troublesome. One or two capsules are usually placed in the cervix. Should the uterine cavity be very large, the capsules may be enlarged by the addition of jackets (Fig. 3 and Tables 1 and 2). Bladder and rectal dosimetry is carried out after each application. Roentgen examination is performed if perforation is suspected. If the capsules are found to be well clustered this damage is improbable.

Vaginal radium was inserted at the first two packings in order to diminish the risk of vaginal metastases. The estimated radiation dose delivered to the vault was 3 000 rad at a depth of 1 cm. Following radium, cases in stage II were usually given supplementary external radiotherapy with ^{60}Co or 34 MeV betatron units: 4 000 rad being delivered to the parametria and the pelvic walls. Cases with advanced disease or large tumor volumes in stages I and II were treated preliminarily with external radiotherapy followed by intracavitary radium

tensive disease contributed to this decision In the remaining cases the uterus could be packed despite the perforation There were no cervical tears

Laceration of the vaginal mucosa attributable to excessive vaginal packing with gauze occurred in two cases (1 %) In one of these, the laceration had to be stitched

Hemorrhage during packing occurred in two cases (1 %) In one of these there was associated perforation of the uterus and a small blood transfusion (400 ml) was given

Fever The presence of radium in the uterus often resulted in a slight rise in body temperature but this did not appear to have any clinical significance Only ten cases (5 %) ran a temperature of 38 C or more for two or more days five were in stage I three in stage II and two in stage IV The fever responded satisfactorily to antibiotic Management was otherwise unaffected None of the patients developed salpingitis However, all those with a past history of salpingitis were given antibiotics for a week as a prophylactic measure

Radiation necrosis of the vaginal vault This complication was noted in two cases (1 %) Intracavitary radium had been supplemented by externad radio therapy in both and the lesions made their appearance after subsequent hyster ectomy They caused little or no trouble and healed spontaneously

Deep venous thrombosis of the leg developed in one case a few days after the second radium application The patient was 75 years old and had had recurring pyometra prior to treatment in addition she had carcinoma of the breast and skin The case was treated with anticoagulants but was otherwise managed according to plan

Late bladder and rectal complications The analysis is of course incomplete as the observation time is short Five cases (2.5 %) complained of bladder trouble one to three years after treatment with cystoscopic evidence of trigonal oedema In two of these necrotic areas in the bladder mucosa were revealed The symptoms were in all instances temporary and responded to simple treatment with urinary antiseptics In four cases (2 %) there were late symptoms from the sigmoid colon or the rectum these were mild and of short duration and no ulcerated areas were seen at sigmoidoscopy

There were no deaths attributable to packing

Discussion

Several authors (1 3 18 21 25) have reported the incidence of residual carcinoma after primary radiation for endometrial carcinoma The superiority

Results

The histologic studies were carried out jointly by the senior staffs of the departments of Pathology and Gynecological Radiotherapy The primary cure rate in the 189 cases with stage I or stage II endometrial carcinoma treated with primary radiation was 174 cases (92 3 %) The 5 year recovery rates are obviously not yet available

The primary cure rate in stage I was 149 cases out of 158 (94 5 %) The histologic specimens in 87 % of the cases presented no traces of malignancy, a hyaline membrane replacing the endometrium Epithelium without signs of vitality was present in 8 % of these cases, and residual viable carcinoma in 5 % Further analysis revealed that the primary cure rate in the 99 cases in which supplementary hysterectomy was performed was 91 % No residual malignancy was present in the specimens obtained from curettage

The primary cure rate in cases in stage II of endometrial carcinoma was 25 out of 31 (80 8 %) Death of intercurrent disease occurred in an additional case one week before control curettage was planned and the effect of radiation could not be judged

Analysis of the complications arising from packing disclosed that these were few and far between and to all intents and purposes without clinical significance The hospital records of the 200 cases of endometrial carcinoma in all stages receiving intracavitary radium were studied, and the following complications were noted A 100 % follow up was maintained

Pyometra Six cases (3 %) had pyometra at the time of admission Packing was carried out as planned, and the condition cleared up spontaneously as treatment progressed In six other cases (3 %), pyometra developed in the course of the treatment, in two of these packing was delayed for a few days while the uterus was drained Apart from this the management was unaffected by this complication

Perforation of the uterus Diagnostic procedures carried out before the treatment began resulted in perforation of the uterus in twelve cases (6 %) Seven of these occurred in local hospitals and five in our department The uterus could be packed as planned in all cases but one in which a tandem was inserted at the first application In nine other cases (4 5 %) the uterus was perforated as a direct result of the packing Eight of these cases were in stage I the other in stage II In one case, a tandem was substituted In another case the perforation necessitated a reversal of the usual time sequence of therapy, and the patient was given external radiotherapy followed by packing, the fact that she had ex

SUMMARY

The preliminary results of a three year investigation of an unselected material of all known cases of endometrial carcinoma in a specific region of the north of Sweden are presented Cases in stage I and stage II were treated with intracavitary radium followed if possible by hysterectomy and bilateral salpingo-oophorectomy Curettage was performed three months after radiotherapy in the remainder The primary cure rate was 95 % in the stage I cases and 81 % in the stage II cases

ZUSAMMENFASSUNG

Es wird uber die vorlaufigen Resultate einer dreijahrigen Untersuchung samtlicher behandelten Falle von Endometrialcarcinom in einem gewissen Bezirk in Nord Schweden berichtet Fälle im ersten und zweiten Stadium der Erkrankung wurden intracavitar mit Radium behandelt und wo moglich erfolgte anschliessend Hysterektomie und beiderseitige Entfernung der Tuben und Ovarien Curettage wurde in den anderen Fallen drei Monate nach der Bestrahlung ausgefuhrt Die primare Heilungsrate war 95 % der Falle im Stadium I und 81 % im Stadium II

RÉSUMÉ

Les auteurs presentent les resultats preliminaires d une etude ayant dure trois ans dans tous les cas non sélectionnes de cancers de l endometre dans une region déterminée du nord de la Suède Les cas aux stades I et II ont éte traités par le radium intracavitaire suivi si possible d une hystérectomie et d'une salpingo oophorectomie bilaterale Un curetage a été fait dans les autres cas trois mois après la radiothérapie Le taux de guerison primaire a éte de 95 % dans les cas du stade I et de 81 % au stade II

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of the packing over the tandem technique has been well documented (1, 18). ARNESON (1), *for example*, stated that the proportion of cases of residual malignancy dropped from 77 to 26 % on changing from the tandem to the packing technique. The present results, a primary cure rate of 95 % in an unselected material with endometrial stage I carcinoma, are much better than any hitherto published. It should be borne in mind that the material is heterogenous with large variations in the differentiation and extension of the growth as well as in uterine size.

Sceptics may query the validity of the histologic studies, both in respect of the number of histologic sections examined and the criteria by which cell vitality has been assessed. We are fully aware that even careful curettage may fail to reveal malignancy lurking deep in the myometrium. Although these considerations may somewhat limit the value of the study, it is hoped that it may provide food for thought. Ever since REYNAUD'S contribution in 1922, the impression has prevailed in many quarters that endometrial carcinoma, being derived from adenomatous tissue, cannot be cured by irradiation. It cannot be disputed that the intrauterine application of multiple sources delivers a lethal radiation dose to most endometrial carcinomas. The application must, however, be carried out properly in order to ensure the delivery of a uniform dose to the entire tumor. Of equal importance is adequate dosage, since a very high irradiation dose is necessary to cure this relatively radioresistent growth. Fortunately, the extreme radioresistance of the uterine walls makes the intracavitary application of such doses feasible.

We do not advocate radiotherapy alone as the treatment of choice for endometrial carcinoma. The fact that failure is occasionally encountered makes supplementary surgery mandatory in the good risk case. Cases with early carcinoma of the highly differentiated type may very well be treated with primary surgery. Their selection is not always so easy, however, and error of judgment may lead to serious consequences. We therefore believe that the standard treatment of all cases of endometrial carcinoma should be primary radiation, supplemented where possible by hysterectomy and bilateral salpingo-oophorectomy. The radiation-induced uterine shrinkage makes operation easier, and the above-mentioned cancerocidal effect diminishes the risk of squeezing tumor cells into surrounding lymphatic and vascular channels at operation. Even if radiation fails to eradicate the growth, there is experimental evidence that irradiated malignant cells dispersed at the time of surgery are less liable to implantation elsewhere. Finally, the adoption of preliminary radiotherapy as a standard measure ensures that all cases of endometrial carcinoma are referred to a therapeutic centre. It is reasonable to suppose that centralization must lead to a degree of diagnostic and therapeutic expertize that cannot easily be obtained in any other way.

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MODIFICATION OF EFFECTS OF PROTON AND GAMMA RADIATION ON THE RECTAL MUCOUS MEMBRANE BY LOCAL HYPOXIA

by

STIG STENSON

The influence of oxygen on the radiosensitivity of living cells is well known (GRAY et coll. 1953, GRAY 1957, FOWLER 1966, RUBIN & CASARETT 1968). It has been shown in a variety of organisms that the anoxic cell is 2 to 3 times less sensitive to radiation of low linear energy transfer (LET∞) than the normally oxygenated cell (ALPER & HOWARD-FLANDERS 1956, DESCHNER & GRAY 1959). The idea to use this phenomenon for protection of healthy tissues when malignant tumours are treated by radiotherapy has been advanced by CHURCHILL-DAVIDSON et coll. (1960) and STECKEL et coll. (1967).

In radiotherapy of carcinoma in the pelvis, the main critical organ in consideration of dose is the rectum (TODD 1938, INGELMAN-SUNDBERG 1947, KOTTMEIER & GRAY 1961). In a recent study (STENSON 1969a), high energy protons and roentgen radiation were found to induce the same type of damage to the rectal mucous membrane but there was a difference in relative biologic efficiency

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Table 1

Semi quantitative comparison of changes in deoxygenated and normally oxygenated segments of rat rectum in groups I—II of proton and gamma irradiated rats

Rectal treatment	Dose rad	Hours after irradiation 40—60 a	40—60 b	60—80 a	60—80 b	80—100 a	80—100 b	100—120 a	100—120 b
Group I proton irradiated (13 rats)									
Norepinephrine	1 400			+	++	+	+	+++	+++
and	2 000			+	++++	+	+++	+	+++
sodium sulfite				0	+	+	+++	++++	+++++
				+	++	+	++		
						+	+++		
	2 400			+	++	++	+++		
				+	++	++	++		
						+	++		
	2 800			+	+				
				+	+				
Group II gamma irradiated (24 rats)									
Norepinephrine	1 500					++	++	+	++
and						+	++	0	++
sodium sulfite						+	++	0	++
								+	++
								0	+
	2 000			+	++	++	+++		
				+	+	+	+		
						++	+++		
						+	++		
						++	++		
	2 500	0	+	++	+++	++	+++		
		0	+	++	+++	+	++		
		0	+	+	++	++	+++		

a = specimen from the deoxygenated part of the rectum b = specimen from the normally oxygenated part of the rectum 0 to 5 + signify different grades of radiation induced changes as defined in a preceding study (Svensson 1969 a)

those animals that were predetermined to survive more than 90 hours The source skin distance was 40 cm the beam axis being perpendicular to the skin (Fig 1) The dose rate was 280 rad/min as determined by a miniature ionization chamber in the rectum The homogeneity of the beam at the rectal level was ± 5 % (Cederlund 1969) The doses were 1 500 to 2 500 rad

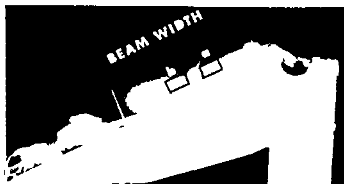


Fig 1 Lateral roentgenogram of rat showing the position at gamma irradiation beam perpendicular to skin *a*=section of rectum giving specimen *a* *b*=section of rectum giving specimen *b*

The possibility of protecting the rectal mucous membrane from radiation damage by creating a state of local hypoxia during irradiation through rectal injection of solutions with vasoconstricting and oxygen reducing substances was demonstrated preliminarily by LARSSON & STENSON (1965) The present paper aims at a closer evaluation of this technique

Material and Methods

Seventy seven inbred Sprague-Dawley female rats weighing 180 to 250 g were used in the study

Proton irradiation Twenty eight rats (group I) were irradiated with a well collimated 187 MeV proton beam (cf LARSSON 1961) The beam was used without intervening absorbers The dose rate was 35 to 125 rad min and the mean LET ∞ at the rectal level 0 5 keV/μm The doses were 1 400 to 2 800 rad and the fluence homogeneity in the rectal area was in the range ± 5 %, determined as described by FALKMER et coll (1959) The rats which should live for less than 90 hours had the whole abdominal cavity uniformly irradiated Those which should live for 90 to 120 hours had, at irradiation, a zone of about 2 cm of the upper part of the abdominal cavity protected This protection permitted the animals to survive the desired period (cf STENSON 1969b) During the irradiation, the position of the animals was checked by means of a television arrangement

Gamma irradiation Forty four rats (groups II—VI) were irradiated with a 3 000 Ci ^{60}Co source (Siemens Gammatron III) The abdominal cavity of the rats was irradiated uniformly except for a 2 cm zone of the upper abdomen of

minutes before irradiation and was continued until the end of irradiation Finally the abdominal incision was re opened the ligature and the catheter removed and the abdominal wall closed

The oxygen reducing treatment in group I (proton irradiated rats) and group II (gamma irradiated rats) started with the infusion of 1 ml norepinephrine and continued after one minute with sodium sulfite solution

The rats in group III received only physiologic saline as rectal treatment, group IV norepinephrine and saline group V only 0 1 mol $Na\ SO_3$ and group VI no rectal treatment at all during irradiation after operation Finally five rats (group VII) received the same rectal treatment as groups I and II but were not irradiated The rectal treatment the type of irradiation and the doses given to the different groups are presented in Tables 1 and 2

The observation periods were 40—60, 60—80 80—100 and 100—120 hours

Histologic technique At the end of the observation periods, the rats were sacrificed by a blow on the head and histologic specimens were taken from that part of the rectum which had been irradiated whilst also having chemical treat ment i e anally to the ligature and thus easily recognized (specimen *a*) Another rectal specimen (specimen *b*) was taken orally to the ligature, i e in irradiated but normally oxygenated region

The same histologic technique for preparation of the specimens was used as in a preceding study (STENSON 1969a) and the criteria for the semi-quantitative histologic grading 0 to V+ were also the same The specimens were about 1 cm in length and sectioned at approximatively 7 μm forming a complete series at planes less than 0 5 mm apart The sections were assessed as unknown in dependently several times by two investigators When the lesions were unevenly distributed in various parts of the intestinal mucosa the final grading was the highest one observed In the 512 assessments of the 128 specimens the variations between different gradings were small on different occasions and between the two investigators Thus the differences were higher than one degree only in one specimen where the grading ranged from 0 to II+ Divergencies in the gradings essentially concerned grades 0 to I+ and I+ to II+

Measurement of oxygen tension The effect of norepinephrine and sodium sulfite on the oxygen tension in the rectum was studied separately in four rats A modified Clark platinum pO_2-electrode (Beckman International Instrument S A) connected for amplifying to a Beckman Physiological Gas Analyzer Model 160 was used (cf GRANGSJO & ULFENDAHL 1962)

The anaesthetized rat was opened with an abdominal incision and put dorsally on an inclined wooden platform with the head uppermost One millimeter norepi

Table 2

Semi-quantitative comparison of changes in specimen a and specimen b of the rat rectum treated with different solutions with and without irradiation of groups III to VII

Experimental groups	Rectal treatment	Dose rad	Hours after irradiation (or treatment) 40—60		60—80		80—100		100—120	
			a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Gamma irradiated										
III 4 rats	0.9 % NaCl	2 000	+	+	++	++	++++	++++	+++	+++
IV 4 rats	Norep. 0.9 % NaCl	2 000	0	++	+	++	+	++	++++	++++
V 4 rats	Sodium sulfite	2 000	+	+	+	++	++	+++	+++	++++
VI 4 rats	0	2 000	++	++	++	++	+++	+++	+++	+++
VII 5 rats	Norepinephrine and sodium sulfite	0	0	0	0	0	0 0	0 0	0	0

a = specimen *a*. *b* = specimen *b*. 0 to V+ signify different grades of radiation-induced changes as defined in a preceding study (STENSON 1969a).

During the irradiation the position of the animals was checked by means of a television arrangement.

Oxygen reducing treatment. The solutions, which were intended to create a state of local hypoxia in the rectal mucous membrane, were norepinephrine (noradrenine 1 mg/ml Astra, Sweden), and 0.1 mol sodium sulfite solution in deionized water, i.e. a slightly hypotonic solution with a pH of approximately 9. They were used in combination or each separately.

Before treatment all the rats were anaesthetized with mebumal sodium (nembutal, Abbott), 4 mg/100 g body weight, given intraperitoneally. A thin rubber catheter, perforated 1 cm from its closed tip, was introduced through the anus 3 to 4 cm into the rectum; the abdomen was opened with an abdominal incision and the catheter was fixed in the rectum by a silk ligature carefully put around the rectum. The abdominal incision was then closed and the animal put dorsally, for treatment, on an inclined wooden platform with the pelvis uppermost (Fig. 1). This position permitted filling of the rectum between the anus and the ligature with the test solution. The rectal treatment was given as a drop infusion 2.7 ± 0.5 ml/min at a pressure of 80 cm H_2O. The infusion started 5 to 6



Fig 3 Photomicrographs of rectal mucous membrane of rat 84 hours after irradiation with 2 500 rad of gamma rays *Left* grade II+ damage of the deoxygenated part of rectum *Right* grade III+ damage of the normally oxygenated part of rectum HEX — eosin × 100

scar One rat was excluded from the study because of the leakage of the rectal treatment to the rectum orally to the ligature

Immediately after irradiation three rats in groups III—IV died from the anaesthetic but these animals were at once replaced with others to make these small groups complete All the rats which died during the planned observation period were excluded from the investigation because of post mortem changes in the rectal mucous membrane

At post mortem examination specimen *a* (chemically treated) and specimen *b* (normally oxygenated) were easily identified There were small haemorrhages in the rectal wall and an opening in the rectal mesentery indicating the place of the ligature at irradiation (Fig 1)

Macro and microscopic examinations Macroscopically, in the entire material there was no obvious damage and no difference between specimens *a* and *b* or between the effects of proton and gamma irradiation

At the microscopic examination no qualitative differences were observed



Fig 2 Photomicrographs of rectal mucous membrane of rat 80 hours after irradiation with 2 000 rad of protons *Left* grade I+ damage of the deoxygenated part of rectum *Right* grade III+ damage of the normally oxygenated part of rectum HTX — eosin × 100

nephrine was infused into the rectum and, after a few minutes, the platinum electrode was put into the rectal lumen When the electrode potential was stable, 0 1 mol sodium sulfite was dropped on the outside of the rectum at the level of the electrode, the electrode potential being recorded every minute Before and after each experiment the electrode was calibrated at 37° C with 0 1 mol sodium sulfite ($pO_2 = 0$ mm Hg) and air saturated water ($pO_2 = 155$ mm Hg)

Results

Nine of the twenty eight rats in group I died during the predetermined observation period Four died from anaesthesia during the first hours after irradiation, one from operative complication and four on the 4th day after irradiation, obviously from radiation induced damage to the intestine In group *II*, three of the twenty eight rats died from operative complications two of them from bleeding into the peritoneal cavity and one from rupture of the abdominal

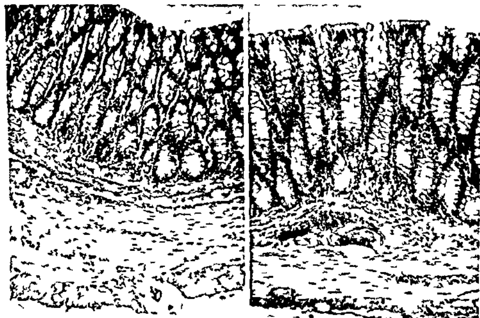


Fig 5 Photomicrographs of rectal mucous membrane of rat 90 hours after treatment with norepinephrine and sodium sulfite for 15 minutes without irradiation Grade 0 damage in both specimen *a* (left) and specimen *b* (right) HTX — eosin × 100

degree of damage in the *a* and *b* specimens In no gamma rat of this observation period was there a difference of 2 grades

The two proton rats irradiated at 2 000 rad and one proton rat irradiated at 1 400 rad in the 100—120 hour period displayed a grade 2 a grade 1, and a grade 0 difference respectively and the five rats gamma irradiated at 1 500 rad differences of 1 or 2 grades

In the rats of group III treated with saline during gamma irradiation there was no difference between the *a* and *b* specimens compared (Fig 4) One rat in group IV treated with norepinephrine and saline during gamma irradiation had no effect of the chemical treatment and in the other rats of this group differences of 1 to 2 grades were noted In group V, treated with 0 1 mol Na_2SO_3 during gamma irradiation a grade 1 difference was noted at the 60—80 80—100 and 100 120 hour periods The rat sacrificed at the 40—60 hour period showed no effect of the chemical treatment At 2 000 rad of gamma radiation the rats of group VI operated upon but without rectal treatment had the same degree of damage in the *a* and *b* specimens. In the five rats of group VII receiving norepinephrine and sodium sulfite for 15 minutes without irradiation there was no reaction in any specimen (Fig 5)



Fig 4 Photomicrographs of rectal mucous membrane of rat 90 hours after rectal treatment with physiological saline during irradiation with 2 000 rad of gamma rays Grade IV+ damage in both specimen *a* (left) and specimen *b* (right) HTX — eosin × 100

between the effects of proton and gamma rays on the rectum The results of the microscopic grading of the damage are given for groups I—II in Table 1 and for groups III—VII in Table 2 In 74 % of all proton irradiated rats of group I (14 out of 19) the changes in specimen *a* were one or two grades lower than in specimen *b*, and in 26 % the grading was the same in the specimens compared The corresponding figures for the gamma irradiated rats of group II were 83 % (20 out of 24) and 17 %, respectively In no assessment was the grade of damage in specimen *a* higher than in that of specimen *b*

In the 40—60 hour period, the three rats gamma irradiated at 2 500 rad all displayed a grade 1 difference in the specimens compared

The 60—80 hour period contained thirteen proton or gamma irradiated rats In nine of them there was a grade 1 difference in three no difference and in one animal (2 000 rad protons) there was a difference of 3 grades between the *a* and *b* specimens compared

Eight proton and eleven gamma irradiated rats were sacrificed 80—100 hours after the irradiation Three proton rats (2 000 rad) displayed a difference of 2 grades (Fig 2), three a grade 1 difference and two no difference between the specimens compared Eight of the eleven gamma rats showed a difference of 1 grade between the specimens compared (Fig 3), and three had the same

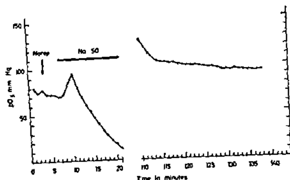


Fig 6 Rat No 1 Diagram showing the changes in oxygen tension in the rectal lumen of rat during treatment with norepinephrine and 0 1 mol sodium sulfite solution

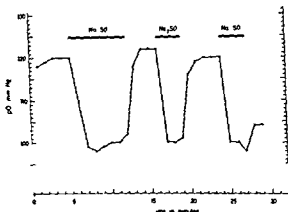


Fig 7 Rat No 3 Diagram showing the changes in oxygen tension in the rectal lumen of rat treated discontinuously with 0 1 mol sodium sulfite solution on the outside of the rectum

In preceding studies (FALKMER et coll 1959 STENSON 1969a) comparison was made between the effects of high energy protons and roentgen rays on rabbit skin and the rectum of the rat In the present study a comparison was made between the effects of protons and gamma rays A dose of 2 000 rad of protons creates approximatively the same histologic effects on the rectal mucous membrane as 2 000 rad of gamma rays (cf Table 1)

The distension of the rectum by filling it with physiologic saline (group III) or the application of a ligature around the rectum containing a catheter (group VI) did not influence the radiosensitivity of the mucous membrane Further

Changes in rectal oxygen tension The following observations were made in four rats subjected to a drop of sodium sulfite solution on the outside of the rectum through an abdominal incision

Rat No 1 had a decrease in oxygen tension from 70 to 80 mm Hg to about 15 mm Hg in 14 minutes after the onset of the sulfite drop The drop was then stopped and the abdominal incision closed After 1 1/2 hour, when the rat was still under anaesthesia, the oxygen tension in the rectum was again measured It soon became stable at 90 to 100 mm Hg (Fig 6) The rat survived the experiment

Rat No 2 reacted similarly but the decrease in oxygen tension was slower than in rat No 1 and this rat died about half an hour after closing of the abdominal incision

In order to examine the possibility of reproducing changes in the oxygen tension in the rectum during the experiment, rats Nos 3 and 4 were subjected to a discontinuous drop of sodium sulfite to the rectum As seen from the curve in Fig 7, the decrease in oxygen tension followed immediately upon the start of the drop, and at the withdrawal the oxygen tension was restored The procedure was repeated twice with the same result A similar result was obtained in rat No 4 In both the latter rats the respiration and the intestinal circulation was seemingly undisturbed during the oxygen measurement

Discussion

The time required for complete renewal of the cell population of the rectal mucosa in the rat has been estimated to about 6 days (BERTALANFFY 1960) After roentgen irradiation of the abdominal cavity with 1 000 rad or more the rat will suffer acute intestinal radiation death within 4 days (QUASTLER et coll 1951) Since the same holds for proton irradiation (STENSON 1969b), the acute intestinal radiation death makes a long term study impossible with the radiation technique used in the present study, as histologic damage cannot be demonstrated in the rectum of rat after 1 000 rad or less (STENSON 1969a) Fortunately, however, the important period after irradiation i e when the changes become serious in connection with the intestinal radiation death, can be studied at 80—100 hours The radiation doses used in the investigation created mucosal changes which could easily be graded histologically

The results indicated that the experimental conditions used permit a study of the radioprotective effects of norepinephrine and sodium sulfite, and it was possible to compare specimens from deoxygenated and normally oxygenated segments of the rectum in each single animal So the possible influence of the individual radiosensitivity was eliminated

RÉSUMÉ

L injection rectale de noradrenaline et de sulfite de sodium pendant l irradiation de l abdomen du rat par les rayons gamma du cobalt ou par des protons de haute energie reduit en partie les graves lesions histologiques constatees sur la muqueuse rectale apres son irradiation L effet protecteur est attribue a l état hypoxique de la paroi rectale du a ce traitement

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norepinephrine and sodium sulfite did not seem to damage the rectal mucosa (group VII)

The results in groups I and II confirm that norepinephrine and sodium sulfite protect the rectal mucous membrane, as previously demonstrated by LARSSON & STENSON 1965 Both norepinephrine and sodium sulfite seemed to contribute to this effect (cf groups IV and V) The rectal treatment in group I seemed to be slightly more effective than that in group II after the same dose, 2 000 rad This may be due to the lower dose rate at proton irradiation giving approximately a 5 times longer treatment period than at gamma irradiation The oxygen reducing treatment was thus applied longer in group I After irradiation at 2 400, 2 500 and 2 800 rad, the difference between compared specimens was less (0 to 1 grade) The doses were apparently too high to permit the same protection as at 1 500 and 2 000 rad

Determination of the oxygen tension could not be made in the thin rectal wall under the experimental conditions of the main experiment By the supplementary technique introduced it was possible to show, however, that norepinephrine and sodium sulfite applied on the outside of the rectal wall, decreased the oxygen tension in the rectal lumen Although proof is still lacking, it may be assumed that the oxygen reduction in the mucous membrane should have been at least as effective when the solution was applied in the rectal lumen, thus directly on the mucosa as when applied on the outside of the rectal wall

The local radioprotective effect of the chemical treatment applied seemed to be due to the hypoxic state of the tissues during irradiation and seemed to be effective for both high energy protons and gamma irradiation A practical consequence of this result might be that rectal damage can be reduced in patients irradiated therapeutically with radiation of low linear energy transfer

SUMMARY

Rectal injection of norepinephrine and sodium sulfite during irradiation of the abdomen of the rat with cobalt gamma rays or high energy protons was found to partially reduce the severe histologic damage of the rectal mucous membrane that is usually seen after irradiation of this structure The protective effect was ascribed to the hypoxic state in the rectal wall induced by the treatment

ZUSAMMENFASSUNG

Bei Bestrahlung vom Bauch der Ratten mit Gammastrahlen und hochenergetischen Protonen wurde Norepinephrin und Natriumsulfid rectal eingespritzt Dabei wurde eine Reduktion der Beschädigung der rectalen Schleimhaut partiell erreicht die sonst bei Bestrahlung dieser Struktur histologisch meistens nachgewiesen wird Es wird angenommen dass die Schutzwirkung eine Folge der Hypoxämie der Rectalwand war die bei der Behandlung hervorgerufen wurde

M RADIUMHEMMET (DIRECTOR PROF J EINHORN) AND THE DEPARTMENT OF
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TUTE OF RADIATION PROTECTION, STOCKHOLM SWEDEN

TFLEGAMMA THERAPY IN CARCINOMA OF THE HYPOPHARYNX WITH SPECIAL REFERFNCE TO YTOLOGICALLY VERIFIED METASTASES IN THE NECK

by

GORAN WERNER and INGVAR BARYD

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Material A total of 195 out of 218 patients referred to Radiumhemmet with carcinoma of the hypopharynx during the period 1955—1965 received treatment and there was an increasing tendency to use high-energy therapy for this group of tumours (Table 1) In 23 patients during the same period their generally poor condition or the spread and advance of the disease precluded any treatment

A total of 83 of the 195 patients treated received ^{60}Co therapy Nine of these

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FROM RADIUMHEMMET (DIRECTOR PROF J EINHORN) AND THE DEPARTMENT OF CLINICAL RADIOPHYSICS (DIRECTOR PROF R WALSTAM), THE NATIONAL INSTITUTE OF RADIATION PROTECTION STOCKHOLM SWEDEN

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Table 3

Patients with carcinoma of the hypopharynx grouped according to site of tumour and survival after ^{60}Co therapy

	Site of lesion					Total numbers of patients	Per cent
	Vallecula epiglottis aryepiglottic fold	Post cricoid area	Pyriform fossa	Pharyngeal walls	Growth into surrounding organs		
Number of patients	28	5	25	7	18	83	100
1 year survival	16/28	2/5	12/25	3/7	9/18	42/83	51
2 year survival	9/28	2/5	10/25	2/7	7/18	30/83	36
3 year survival	7/27	2/5	4/22	1/7	4/18	18/79	23
More than 5 year survival	6/24	1/4	3/16	—	1/15	11/65	17

Of the eleven patients who survived 5 years or more nine had growths in the upper hypopharynx and two in the lower hypopharynx

Table 4

Distribution according to location of tumour in upper or lower part of the hypopharynx

	Total number of patients	Women		Men	
		Number	Per cent	Number	Per cent
Carcinoma of the upper part of the hypopharynx	63	10	16	53	84
Carcinoma of the lower part of the hypopharynx	12	8	67	4	33
Tumours with extended growth in both the upper and the lower parts of the hypopharynx	8	2	25	6	75

Co therapy as it became possible to treat increasing numbers of patients with high-energy technique (Table 1) Sixty four of these were men and nineteen, i e 23 %, were women (Table 2) This distribution between the sexes is markedly different from that which existed during the period 1939—1947 when JACOBSSON (1951) reported the results of roentgen treatment i e in a total of 322 patients of whom 119 were men and 203 women

The mean age on admission in the present series of patients was 61 years

Table 1

Type of treatment given in the different periods

Year of admission	Treated with roentgen	Treated with ^{60}Co	Not accepted
1955	19	3	3
1956	14	5	3
1957	32	6	2
1958	7	11	—
1959	5 (7)	9	3
1960	13	6	5
1961	8 (10)	9	3
1962	3	13	2
1963	2	5	—
1964	5	7	—
1955—1965	108 (112)*	83	23

* Four patients were operated upon before roentgen therapy

Table 2

Distribution according to age and sex of the 83 patients who received ^{60}Co therapy — The mean age of the men was 61 and that of the women 59 years

Age in years	Men	Women
30—39	2	—
40—49	9	2
50—59	13	8
60—69	27	6
70—79	13	3
Total number 83	64 (77 %)	19 (23 %)

were also subjected to therapy with 15 MeV electrons or 180 kV roentgen treatment. Sixty-five of the eighty-three patients were treated with kilocurie techniques and eighteen with a short distance gamma beam, the so called decacurie technique. The remaining 112 patients received roentgen irradiation.

Some degree of selection can be assumed to have taken place, inasmuch as the eighty-three patients who received high energy therapy were considered prognostically more favourable than those who had roentgen irradiation during the same period. This was certainly true in the eighteen patients who received decacurie therapy, and subsequently to a lesser extent in the patients given

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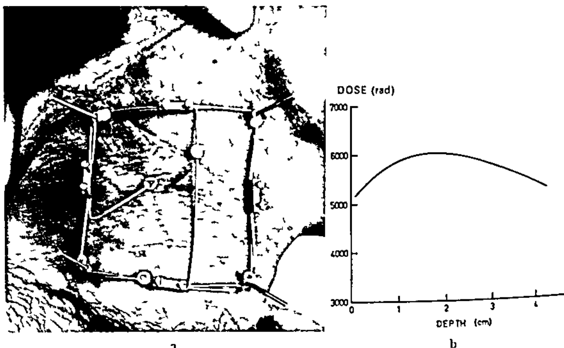


Fig. 1. Technique for decacurie treatment. a) A wire jig with four axial pins giving the directions of the central rays and the points for depth dose calculation. b) Example of depth dose at point A on skin in (a). 4 000 rad was given to each one of the four fields.

for the men and 59 years for the women. Only thirteen of the patients were under 50 and none was over 74 years old.

The series was divided into groups of patients according to the origin and location of the tumour (Table 3) and further subdivided into two groups, representing respectively carcinoma of the upper and lower parts of the hypopharynx (Table 4), drawing the borderline at the superior margin of the cricoid cartilage. Thus the first group includes carcinomas situated mainly in the external parts of the larynx and the piriform fossa; all the others referred to were carcinomas of the lower part of the hypopharynx, except eight patients in whom it was impossible to judge whether the tumour originated in the upper or lower parts of the hypopharynx. The reasons for this division were differences in the sex distribution and predisposing factors. Of the 83 patients treated, 63 had growths that had arisen in the upper part of the hypopharynx. The tumour had invaded adjacent organs such as the oesophagus or the base of the tongue in eighteen patients; the extent of the tumour in eight of these made it impossible to determine whether it had arisen from the upper or lower parts of the hypopharynx.

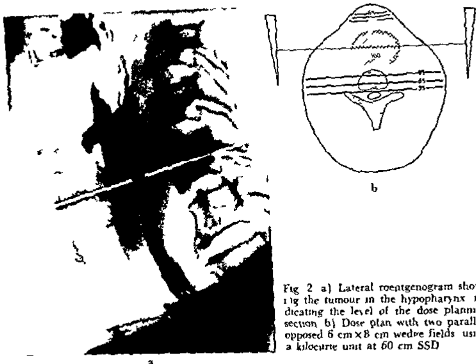


Fig 2 a) Lateral roentgenogram showing the tumour in the hypopharynx indicating the level of the dose planning section b) Dose plan with two parallel opposed 6 cm × 8 cm wedge fields using a kilocurie unit at 60 cm SSD

All the neoplasms in this material were histopathologically verified as squamous-cell carcinomas mostly of a relatively poorly differentiated solid type

Fifty two patients had demonstrable unilateral and nine patients had bilateral lymphatic gland metastases in the neck The occurrence of these metastases was checked by cytologic investigation after aspiration biopsy (FRANZEN 1968) and additionally in five patients by extirpation of the gland and P A D Multiple primary malignant tumours were present in seven patients in three of these another primary malignant tumour had arisen prior to that in the hypopharynx and in four patients other primary malignant tumours occurred during the follow up period Eighteen (22 %) of the patients had had their first symptoms more than six months prior to admission to Radiumhemmet in the others the period was less than six months

Methods of examination The size of the tumour was always determined by roentgen examination of the soft parts of the neck and contrast roentgen examination of the hypopharynx Indirect laryngoscopy and hypopharyngoscopy

Table 5

Tumour dose and fractionation times in relation to 2 year survivals

Tumour dose in rad	Fractionation time in weeks						
	<5	5	6	7	>7	Total number	Per cent
<5 500	0/2	3/3		2/2		4/7	57
5 500—5 999	2/6	0/5	0/2	0/1		2/14	14.3
6 000—6 499	6/9	4/9	6/13	1/5	0/2	17/38	44.7
6 500—6 999	0/3	1/3	0/5	1/2	3/5	5/18	27.7
>6 999	0/1		0/2	0/1	2/2	2/6	33
Total	8/21	7/20	6/22	4/11	5/9	30/83	36

were usually also used to determine the margins of the growth These methods were combined with biopsy of the primary tumour Thin needle aspiration biopsy (FRANZÉN 1968) was always carried out when lymphatic gland metastases were possibly present in the neck Direct extension of the growth into the regional lymphatic glands in the neck sometimes occurred when the primary tumour was advanced It was then often impossible to determine whether or not the changes were due to lymphatic gland metastases The doubtful cases were included among the lymphatic gland metastases

Irradiation of these tumours was until 1955 carried out almost exclusively with conventional roentgen irradiation Between 1955 and 1960 some patients with carcinoma of the hypopharynx were treated with ^{60}Co, employing the short distance gamma beam technique Subsequent to 1960 most of the patients were irradiated with the kilocurie technique

Short distance gamma beam technique (decacurie technique) Methods based on directing a number of circular radiation beams onto the tumour region from a radium source at a distance of 6 to 10 cm were developed The technique was further developed when ^{60}Co sources with activities of 10 to 50 Ci (decacurie sources) for this type of therapy and more favourable dose distributions could be achieved (WALSTAM 1965) Dose calculations and duplication of the positions of the treatment fields were based on the production of individual wire jigs These jigs were so designed that they followed the skin surface in the treatment area and could be fitted with axial pins for the central rays of the radiation beams and with indicators at the points selected for depth dose calculations (Fig 1 a)

The percentage contribution from each field was obtained for each of the

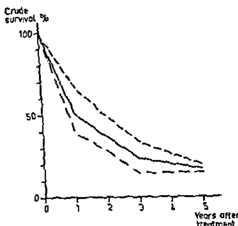


Fig 3 Survival curves for the 83 patients with carcinoma of the hypopharynx who received ^{60}Co therapy The total 5 year survival rate was 17 (———) The 5 year survival rates for 52 patients with cytologically verified lymph node metastases (- - -) and for 31 patients without verified lymph node metastases (— — —) were respectively 15 % and 20 %

calculation points selected by rotating transparent flexible isodose diagram around the axial pins Multiplication by the loading on each field and summation over all the fields gave the dose for each calculation point The resultant depth doses for the points selected on the skin surface are illustrated by the depth dose diagram in Fig 1 b The central rays of the beams were made to coincide with the directions of the axial pins of the wire jig and the entrance points were marked on the skin By comparing the skin markings with the axis prior to each treatment it was possible to ensure that the patient maintained the correct position and that the skin markings did not change position, for instance due to reduction in the size of the tumour an effect which could result in considerable overdoses in certain areas The number of fields depended on the extent of the tumour The treatment was in general 1 000 rad to one field a day Each field was irradiated with about 4 000 rad to achieve a tumour dose of 6 000 rad The average treatment time was 30 days

The kilocurie technique To determine the size of the tumour and the position of the spinal cord a lateral roentgenogram was obtained for each patient in supine position and an enlargement indicator was inserted (Fig 2 a) The position of growths with considerable vertical extension was determined from two or more sections in each of which a strip of dental impression compound (Kerr) was cast to indicate the contours A mask of the outline of the neck was made at the same time to duplicate the treatment position

Dose planning was always carried out with two diametrically opposed fields provided with wedge filters in order to achieve uniform dose distributions in the affected region (Fig 2 b) When planning for several sections the tumour

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The percentage contribution from each field was obtained for each of the

Table 6

Survival after treatment of carcinoma of the hypopharynx

	With lymph node metastasis of the neck (5? patients)	Without lymph node metastasis of the neck (31 patients)
1 year survival	?2/52 (40)	21/31 (68)
2 year survival	15/52 (29)	15/31 (48)
3 year survival	7/49 (14)	10/30 (33)
More than 5-year survival	6/50 (15)	5/25 (20)

Table 7

Survival after treatment of carcinoma of the hypopharynx with different techniques

	Decacurie (18 patients)	Kilocurie (65 patients)
1 year survival	10/18 (56 °)	32/65 (49 %)
2 year survival	6/18 (33)	24/65 (37 °)
3 year survival	3/18 (17 °)	14 61 (23 °)
More than 5-year survival	3/18 (17)	8/47 (17)

eighty three patients teated with ⁶⁰Ca 27 patients were subject to operation as follows

Tracheotomy (in a total of 16 patients)	
Before treatment	4
During or less than 2 months after treatment	1
More than 2 months after treatment	11
Neck dissection (in a total of 9 patients)	
Before treatment	1
During or less than 2 months after treatment	1
More than 2 months after treatment	7
Gastrostomy (in a total of 11 patients)	
Before treatment	2
During or less than 2 months after treatment	4
More than 2 months after treatment	5
Laryngectomy	
In all cases more than 2 months after treatment	6

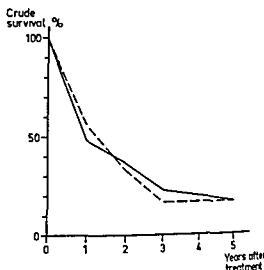


Fig 4 Survival curves for 18 patients treated with the decacurie technique (– – –) and 65 patients treated with the kilocurie technique (———) Two thirds of the patients in each group had cytologically verified lymph node metastases before receiving therapy and the 5 year survival rate for both groups was about 17 %

dose in each section was normalised to the tumour dose in the section through the centre of the tumour The reference doses and the planned values were checked with small condenser ionisation chambers The central doses for these sections could be determined by measuring the entrance and exit doses for various sections

The treatment was given with a Siemens Gammatron I in the range 2 500—1 500 Ci (HULTBERG et coll 1959) The collimator consisted of a number of tungsten plates which could be introduced into the beam individually in order to obtain irregular beams and to shield particular areas for instance, the larynx

Before the first treatment was given, check films of the treatment area were exposed, using the cobalt apparatus This checking is now performed with a roentgen simulator Prior to each treatment and in the course of the treatment, particularly when changes have been made the limits of the fields are controlled by fluoroscopy and a roentgen film with the simulator

A tumour dose between 6 000 and 6 500 rad was usually given (Table 5) over 5 to 6 weeks to one field a day and with a daily tumour dose of between 200 and 250 rad Mucosal reaction of the hypopharynx was generally observed 2 to 4 weeks after the commencement of treatment At the end of treatment and during the weeks immediately following, a moderate epithelial reaction over the affected area was observed The patients under treatment received comprehensive diet, generally fortified with vitamin injections and when sideropenia occurred, iron injections A number of patients were also given antibiotic protection during a period immediately subsequent to treatment It was usually possible to avoid acute oedema and thus tracheotomy or gastrostomy In the

Table 8

Survival after treatment of carcinoma in different parts of the hypopharynx

	Upper hypopharyngeal carcinoma (63 patients)	Lower hyperpharyngeal carcinoma (12 patients)	Carcinoma of both parts (8 patients)
1 year survival	32/63 (51)	7/12 (58)	4/8 (50 %)
2 year survival	22/63 (35)	4/12 (33)	4/8 (50 %)
3 year survival	12/59 (20)	3/12 (25)	2/8 (25)
More than 5 year survival	9/49 (18)	1/8 (13)	1/8 (13)

one of these patients survived for more than 6 years and three for more than 2 years after the radiation treatment All the nine patients are now dead the cause of death in all instances being malignancy

The 5 year survivals for the patients without lymph node metastases was thus 20 % and for those with confirmed lymph node metastases 15 %

No definite differences in survival rates could be noted in this series between patients who received decacurie treatment and those who had kilocurie treatment as may be seen from Fig 4 and Table 7

The three patients surviving more than 5 years have died of *intercurrent* disease 8 to 9 years after the ^{60}Co treatment

The results of the treatment of the patients with tumours situated in the upper and lower parts of the hypopharynx, respectively are summarized in Fig 5 and Table 8 For the patients with tumours in the upper part of the hypopharynx the 5 year survival rate was approximately 18 % in the other groups it was smaller

Discussion

The previously mentioned predisposing factors were sideropenia the Plummer Vinson (Patterson Kelly) syndrome and misuse of alcohol and tobacco Few of these factors previously so *significant* in Sweden were recorded in the present material The Plummer Vinson syndrome is a condition occurring predominantly in middle aged women *rarely in* men Anaemia is common unless sideropenia is present Our material included only 19 women most of whom were elderly Six women and five men, however had a moderate degree of sideropenia with serum iron values between 0 015 and 0 089 mg% at the commencement of treatment A more typical Plummer Vinson condition with dysphagia atrophic changes in the buccal glossopharyngeal and oesophageal membranes as well as spoon shaped nails were noted in some of these patients

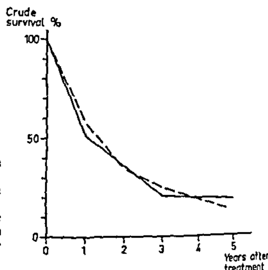


Fig 2 Survival curves for 63 patients with carcinoma of the upper part of the hypopharynx (———) and for 12 patients with carcinoma of the lower part of the hypopharynx (– – –) The 5 year survival rate for the patients with tumours originating in the upper part was about 18 % and for those with tumours in the lower part of the hypopharynx was about 13 %

Each patient was examined by a radiotherapist and an otolaryngologist before radiation treatment was commenced All the patients had regular follow up examinations at Karolinska sjukhuset, with intervals between 2 and 6 months up to June 30th 1968 No patient was missed in the follow up and for 65 of the patients treated during 1963 or earlier the follow up was at least 5 years

Results

The results for all the patients treated with ^{60}Co are summarized in Fig 3 The 5 year survival in the whole series was about 17 % Nine patients died of intercurrent disease, four of them within 5 years of treatment The total 5 year survival rate after correction for the mortality due to intercurrent disease was approximately 31 % Two of the eleven patients who were alive 5 years after the treatment were known to be suffering from recurrences or metastases and these were removed by operation, in one patient the operation took place at 1 and 3 years and in the other patient 6 and 8 years after the primary radiation therapy Nine of the eleven survivors had no known recurrences or metastases when examined 5 years after treatment one had suffered a relapse and another patient had a fresh primary tumour

The survival times for the patients without and with verified lymph node metastases in the neck at the commencement of treatment are given in Fig 3 and Table 6

One of the six patients who survived more than 5 years had bilateral lymph node metastases before treatment Nine patients had bilateral cervical metastases,

were 64 men and 19 women It is particularly interesting that for the 52 patients with lymph node metastases confirmed by cytologic examination, the 5 year survival rate was 15 % No equivalent material has yet been reported for hypopharyngeal tumours in the neck

In earlier material from the same clinic, when roentgen irradiation was used there is no record during the period 1939—1942 of any patient with metastases in the neck who survived as long as 4 years During the period 1943—1947 there were only four patients out of 165 treated with conventional roentgen radiation who survived 4 years or more LEDERMAN (1962) and LEDERMAN & MOULD (1968) reported a 5 year survival rate of 7 % for patients with lymph node metastases In our material the 5 year survival rate for patients with confirmed lymph node metastases was 15 % (see p 139) This was not much lower than for patients without confirmed metastases in the neck (20 %, see p 139 and Fig 3) It should be noted that only one of the patients who survived over 5 years in spite of cytologically confirmed lymph node metastases before treatment belonged to the group which was operated upon during the follow up period Although the number of patients was small the improved result can to a large extent be attributed to the ^{60}Co treatment However one must bear in mind the fact that as a result of better diagnosis smaller metastases can be shown and verified and that an increasing number of prognostically favourable cases therefore come into the group with confirmed metastases In the present material lymph node metastases in the neck were not an excessively unfavourable sign when the method of treatment was the one described One of the nine patients with bilateral cytologically confirmed mestastases in the neck survived without relap e for 5 years after treatment

The development of puncture cytology has also implied that in the frequent follow up studies that took place in this material it was possible to reveal recurrences at a much earlier stage than was possible previously This has increased the possibility of using radical surgical methods to save these patients For the 2 year survival the results were not dependent on the dose or frac tionation times used (see Table 5) This has also been reported by other workers CLEMENT et coll 1964) see also the results reported by KUTTIG (1963) and WOOD (1959) — It is difficult in our material to draw any conclusions from the comparison between the patients treated with the decacurie technique and those treated with the kilocurie technique Those who were treated according to the former technique were pecially selected although twelve of the eighteen patients had confirmed lymph node metastases Of the sixty five patients treated with the kilocurie technique there were forty who had cytologically confirmed lymph node metastases prior to the commencement of radiation therapy Thus two-thirds of those who had decacurie therapy as well as those who received

Dietetic factors are of importance in the development of the Plummer Vinson syndrome (JACOBSSON 1961, WYNDER et coll 1957) Iron and vitamin C deficiency has been fairly common among women in certain parts of Sweden and this syndrome has often been encountered in the northern regions Great improvements in the general diet has occurred in recent decades in Sweden however Since 1938 all white flour has been fortified with iron and vitamin B complex These prophylactic measures have probably been successful in reducing the incidence of the Plummer Vinson syndrome and thus carcinoma of the hypopharynx

Carcinoma of the lower part of the hypopharynx is especially common with the Plummer Vinson syndrome About 75 % of the hypopharyngeal carcinomas in the previously published series from this hospital, were situated at the lower part of the hypopharynx Only 12 of the 75 carcinomas in the present series were in this location In eight patients (Tables 3 and 4), however, the tumour was growing at both the upper and lower parts of the hypopharynx

As regards tobacco and alcohol the records were less certain There were, however, seven clear cases of ethylism all in men The composition of the patient series was relatively unfavourable, with 25 tumours arising in the sinus piriformis, 18 large tumours that invaded adjacent organs, and 52 of the 83 patients had confirmed lymph node metastases prior to treatment However, the total 5 year survival was approximately 17 % A total of 84 % of the patients with carcinoma of the upper hypopharynx were men and 67 % of those with carcinoma of the lower hypopharynx were women This corresponds well with the material previously reported from other clinics (JACOBSSON 1951) It should be pointed out first that carcinoma of the upper part of the hypopharynx occurred mostly in men, in whom the prognosis is always considered worse than in women, and secondly that the tumours were often extensive at the commencement of treatment The latter was usually due to the fact that tumours arising in the piriform fossa often present few symptoms and therefore reach a more advanced degree of growth before diagnosis

For a comparison with the results previously reported by JACOBSSON (1951) from Radiumhemmet it must be borne in mind that the composition of the material has changed over the years During the years 1939 to 1947 the tumours in about three quarters of the patients were located in the lower part of the hypopharynx while in 63 of the 83 patients in the present series the tumours were in the upper part of the hypopharynx This is undoubtedly connected with alterations in the etiology of the tumours described above and with the distribution between the sexes In the period 1939—1947 there were 119 men and 203 women, while in the series treated with ^{60}Co during the period 1955—1965 the distribution between the sexes was reversed i e there

l hypopharynx superieur environ de 18 % Le taux de survie pour 59 malades avec de metastases du cou verifiees cytologiquement etait de 15 % Le pronostic etait defavorable pour cette serie qui comprenait 64 hommes et 19 femmes dont l age moyen etait d environ 60 ans

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kilocurie treatment had verified lymph node metastases (see Fig. 4).

Preliminary reports on small selected materials have recently indicated a new way to improve the prognosis. For instance, SILVERSTONE et coll. (1963), DALBY (1964), HUDSON & CAVANAUGH (1965) give as definite planned treatment a 5 500 rad tumour dose with ^{60}Co over a period of 5 weeks followed by radical surgery within 3 to 6 weeks. They have reported a 2 year absolute survival rate of 72 % and this must be considered a remarkably good result even for a small selected material. CUNNINGHAM & CATLIN (1967) maintain that radiation therapy alone cannot cure advanced pharyngeal carcinoma but that combinations of surgery and radiation therapy may save a few cases not controllable by any one of the methods alone.

Primary radical surgery, which has been prescribed by some workers, has considerable disadvantages in the treatment of hypopharyngeal tumours. Even if the average age involved is comparatively high, and in spite of the fact that ^{60}Co therapy can be quite trying for the patient, no mortalities during the treatment have been recorded. With laryngectomy, with or without neck dissection, on the other hand, the operation mortality is comparatively high and the consequences for the patient are more severe.

SUMMARY

An analysis of a total of 83 patients with hypopharyngeal carcinoma treated with ^{60}Co during the period 1955—1965 is presented. The treatment methods are discussed. The total 5 year survival rate was approximately 17 % and in patients with upper hypopharyngeal carcinoma it was about 18 %. The survival rate was 15 % for 52 patients with cytologically verified metastases in the neck. The series was prognostically unfavourable with 64 men and 19 women and an average age of about 60 years.

ZUSAMMENFASSUNG

Eine Analyse von 83 Patienten mit Karzinom des Hypopharynx, die in den Jahren 1955—1965 mit ^{60}Co behandelt wurden, wird vorgelegt. Die Behandlungsmethoden werden besprochen. Die totale 5 Jahre Überlebenszeit war ungefähr 17 % und in Patienten mit Karzinom des oberen Teiles des Hypopharynx 18 %. Die Überlebensrate war 15 % in 52 Patienten mit zytologisch bestätigten Metastasen im Hals. Die Serie war prognostisch ungünstig mit 64 Männer und 19 Frauen und einem Durchschnittsalter von ungefähr 60 Jahren.

RÉSUMÉ

Un total de 83 malades atteints de cancer de l'hypopharynx a été traité par le ^{60}Co au cours de la période 1955—1965. Les auteurs discutent les méthodes de traitement. Le taux de survie total à cinq ans était approximativement de 17 % et pour le cancer de

Table 1

International comparisons or calibrations of secondary standards of other countries with the DAW cavity ionization chambers

Country	Place of measurement	Institution	Time	Type of measurement
Hungary	Budapest	Oncological Institute Budapest	July 1964	Measurement of ^{60}Co Unit Gravicert
Bulgaria	Berlin	Oncological Institute Sofia	Jan 1965	Calibration of Siemens dosimeter unit
CSSR	Prague	Institute for Nuclear Research Prague	May 1965	Measurement of Co source for the high pressure chamber to be constructed
CSSR	Berlin	Institute for Nuclear Research Prague	Nov 1966	Calibration of two chambers as secondary standards
Hungary	Berlin	State Board of Metrology Budapest	Nov 1966	Calibration of two chambers as secondary standards

deleeff Institute Leningrad U S S R (VNIIM) These comparisons are of special interest for two reasons Firstly they enable a comparison to be drawn between two methods of determining exposure i e the free air and cavity ionization chambers Secondly because a large number of countries have had their secondary standards calibrated by one or other of the centres reporting this intercomparison For instance DAW cavity chambers have been used to calibrate commercial chambers of Czechoslovakian Hungarian and Bulgarian institutions as secondary standards because these countries still lack the facilities for absolute measurements (Table 1) Furthermore NPL standard chambers have been compared with primary standard chambers of the NBS Washington (WYCKOFF et coll 1963) NBS have calibrated a Swedish secondary standard and so on So these comparisons will give some idea of the accuracy of the roentgen unit as used in a larger number of countries

Ionization chambers for the absolute determination of exposure

The cavity ionization chambers of the Institute of Biophysics of DAW Berlin Buch The realization of the unit of exposure in the Institute of Biophysics of DAW is accomplished with a number of graphite cavity chambers with effective

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INTERNATIONAL COMPARISONS OF IONIZATION CHAMBERS FOR ABSOLUTE MEASUREMENTS OF THE ROENTGEN AT PHOTON ENERGIES OF ABOUT 1 MeV

by

A Rakow, W Weil, M Yudin, G Ostromuchova, R Kononova
A R S Marsh and J W G Dale

Accuracy of dose determination is of great importance for many applications of ionizing radiations in radiation therapy radiobiology, and radiation biophysics The absorbed dose (rad) is at the moment generally determined by measuring the ionization in air and multiplying this by a conversion factor For example in the U K there is a code of practice for the dosimetry of high energy roentgen and gamma ray beams which gives conversion factors for particular types of chambers calibrated with 2 MV roentgen rays (Hospital Physicists Association 1964) Calibrations of commercial ionization chambers designated for use as secondary standards are carried out at regular intervals by the various state organizations using their own particular radiation standards

The purpose of this paper is to report a direct comparison between the cavity ionization chambers of the Institute of Biophysics of the German Academy of Sciences of Berlin, G D R (DAW) with those of the National Physical Laboratory, Teddington U K (NPL) and with the pressure chamber of the Men

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by means of plastic inserts so that a reproducibility of the control readings within ± 0 1 per cent is attained The ionization measured per unit mass of dry air was corrected by a correction factor of the form

$$k = k_{Br}\ k_{S\,h}\ k_L\ k_{St}\ k_S$$

where

k_{Br} = correction factor for the different electron stopping power of the chamber wall material and air

$k_{S\,h}$ = correction factor to take into account the attenuation of the primary radiation in the chamber wall

k_L = correction factor to take into account the non uniformity of the primary radiation intensity over the active chamber volume through wall attenuation and through geometric attenuation

k_{St} = correction factor to take into account scatter from the chamber stem

k_S = correction factor for extrapolation to complete saturation

These corrections so far have been determined for the experimental conditions existing at DAW Berlin Buch (RAKOW & WILL 1963) For the conditions at NPL Teddington and VNIIM Leningrad some of the corrections could be determined directly by experiment and the shift of the remaining corrections could be estimated

The comparisons were carried out with one of the DAW graphite cavity chambers which permits absolute measurements of the exposure and with a secondary standard chamber of air equivalent material (WILL & RAKOW 1970) Table 2 gives data of interest about these chambers Table 3 contains corrections introduced into the measuring values of the graphite chamber under various conditions of irradiation

Cavity ionization chambers of the NPL Teddington The primary standard in the U K for exposure measurements with 2 MV roentgen rays is based upon three cylindrical graphite cavity chambers of nominal volume 2 cm³ (BARNARD AXTON BELCHER & MARSH 1956) The chambers are mounted integrally on duralumin stems permanently connected to lengths of non microphonic co-axial screened cable The polarizing potential applied to these chambers is 300 V and their wall thickness is 500 mg/cm

The chambers in turn are connected to one of a pair of Townsend balancing and measuring systems incorporating an electrometer triode valve bridge and standard capacitor A whole beam parallel plate monitor chamber is permanently connected to the other system

Table 2

Details of the two DAW-chambers used for the intercomparisons

Chamber	C 1 5 No 2	H III/1 5 No 1
Measuring volume	1 507 cm³	About 1 5 cm³
Material	Graphite	Air equivalent material
Wall thickness	3 4 mm = 580 mg/cm²	3 4 mm = 590 mg cm²
Chamber voltage	400 V	400 V
Sensitivity according to DAW calibration at t=22 C p=760 mm Hg RH=0 % complete saturation	4 60 × 10⁻¹⁰ C/R	4 91 × 10⁻¹⁰ C/R

Table 3

Correction factors for the DAW cavity chamber C 1 5 No 2 under different conditions of irradiation

		Corrections for the conditions of irradiation at		
		DAW	NPL	VNIIM
k_{Br}	Stopping power	1 004	1 004	1 004
k_{Sch}	Attenuation by total wall thickness	1 015	1 015	1 015
k_U^{Sch}	Irregularity of the intensity by attenuation	0 997	0 997	0 997
k_U^S	Irregularity of the intensity by geometry	0 995	0 999	0 995 (SCD=60 cm)
k_{St}	Stem scatter	0 997	0 997	0 997
k_S	Saturation	1 003	1 001	1 000
Total	correction factor	1 011	1 013	1 008 ± 0 010

Maximum uncertainty of absolute exposure determination ± 2

volumes of from 1 cm³ to 2 cm³, the design of which has already been described (RAKOW & WILL 1963) The wall thickness of the chambers is varied by adding or removing close fitting graphite caps, for ^{60}Co γ radiation the thickness is 580 mg/cm During measurement the chambers are connected via a co-axial cable to an electrometric photocell compensator (Townsend compensator), whose measuring capacitor, as well as the precision voltmeter and the thermometer and barometer for air density determination have been calibrated by the DAMW (DAMW = Deutsches Amt fur Meßwesen und Warenprufung) The chamber polarizing potential is 400 V The constancy of chamber sensitivity is checked by a ^{90}Sr check source in which the constant location of the chambers is ensured

therefore a value 1 022 has been used in the comparisons now reported This is close to the value 1 020 used in an international comparison in 1961 (ICRU 1964)

Estimates of the individual factors are given in Table 4 For the last correction factor f_r mutually compensating terms are thought to make its most likely value 1 000 but this factor has the greatest degree of uncertainty associated with it The present estimate of the limits of uncertainty in the overall correction factor 1 022 is ± 0 008 The NPL wish to point out that they are at present reviewing the values of these factors and intend to publish new estimates when the work has been completed

High pressure standard ionization chamber of the Mendeleeff Institute for Metrology (Leningrad) The primary standard of the U S S R for the energy range from 0 3 MeV to 3 MeV is a parallel plate high pressure ionization chamber with aluminium strips as potential guides The high voltage plate is 75 cm long the collecting electrode 25 cm long and the two guard electrodes each 30 cm long All electrodes are 40 cm wide and the plate separation is 40 cm The beam entrance window consists of 5 77 mm thick duralumin The design of the chambers was described by AGLINTSEV et coll (1961) The air pressure within the chamber may rise to a maximum of 20 atmospheres and the chamber voltage to as high as 15 kV The ionization current is measured by Townsend compensator

Ionization measured per unit mass of air is corrected by the following factor

$$K = K_1 \; K \quad K_3 \; K_4 \; K_5 \; K_6$$

where

K_1 = correction for radiation attenuation in the inlet window of the tank
K_2 = correction for radiation attenuation by air layer between the inlet window of the tank and the centre of the measuring electrode
K_3 = correction for absence of saturation conditions in the standard chamber
K_4 = correction for penetration of radiation through the diaphragm edges
K = correction for displacement of the effective region of gamma radiation absorption relative to the measuring volume
K_6 = correction for influence of the scattering radiation in air

The magnitudes of the individual factors are listed in Table 5

Sources of radiation

The ⁶⁰Co teletherapy unit of DAW Berlin Buch The measurements in Berlin Buch were carried out on the Theratron Junior ^{60}Co irradiation unit of the

Table 4

Correction factors for the NPL 2 cm³ standard graphite cavity chambers for 2 MV roentgen rays

$\frac{(m'en)_{air}}{(m'en)_{carbon}}$	0.999
$S_{m\,air}^{\ carbon}$	1.003
f_w	1.018
f_e	1.002
f	1.000
Total correction factor	1.022 ± 0.008

Maximum uncertainty of absolute exposure determination ±1.5 %.
For the NPL calibration service the value of f is taken as 1.008 in order to retain a total correction factor of 1.03 as discussed on p. 148.

An exposure measurement in roentgens by the NPL chambers is given by

$$I\left[\frac{(m''en)_{air}}{(m''en)_{carbon}}\,S_{m\,air}^{\ carbon}\;f_w\,f_e\,f\right]$$

I = charge in coulombs collected per 2.58×10^{-4} kg of dry air

$\frac{(m''en)_{air}}{(m''en)_{carbon}}$ = ratio of the mass energy absorption coefficients

$S_m{}^{c}_{a}$ = stopping power ratio carbon to air including polarization effect

f_w = correction factor to take into account the attenuation due to the presence of the chamber wall

f_e = correction factor to take into account lack of electronic equilibrium

f = composite correction factor to take into account lack of saturation, configuration factors and stem scatter contribution etc.

When the NPL initiated their 2 MV calibration service in 1956, the overall correction factor (the value of the expression in square brackets) was taken to be 1.03. However, by the year 1959 evidence had accumulated that this was probably an overestimate possibly by as much as 1 per cent (BARNARD & MARSH 1959, BARNARD, MARSH & HITCHMAN 1964). Nevertheless, the value 1.03 has so far been retained for routine calibrations in the interests of consistency, bearing in mind that it still lies within the limits of uncertainty associated with the value now thought to be more nearly correct. There is no reason however, why, in international comparisons a more recent estimate should not be taken, and

Table 6

Results of the comparison of the cavity ionization chambers of DAW and NPL

Chamber		C 1 5 No 2	H III/1 5 No 1
Sensitivity as determined by NPL (uncorrected) C/R × 10	(a)*	4 639**	4 934
	(b)	4 640	4 935
	(c)	4 641	4 931
	Mean	4 640	4 933
Sensitivity as determined by NPL (corrected) C/R × 10		4 54	4 83
Sensitivity as determined by DAW (uncorrected) C/R × 10		4 65	4 97
Sensitivity as determined by DAW (corrected by DAW for NPL conditions) C/R × 10		4 59	4 90
Ratio of uncorrected sensitivities NPL/DAW		1 00	0 99
Ratio of corrected sensitivities NPL/DAW		0 99	0 99

* The comparison was carried out on three separate occasions

** All sensitivities quoted are for dry air at 22 C and 760 mm Hg

Table 7

Results of the comparison of the cavity chambers of DAW with the high pressure ionization chamber of VNIIM

Chamber		C 1 5 No 2	H III/1 5 No 1
Sensitivity as determined by VNIIM C/R × 10	(a)	4 54**	4 93
	(b)	4 49	4 87
	(c)	4 58	
	(d)	4 63	
	Mean	4 56	4 90
Sensitivity as determined by DAW (corrected by DAW for VNIIM conditions) C/R × 10		4 60	4 91
Ratio of sensitivities VNIIM/DAW		0 99	1 00

* The comparison was carried out on four separate occasions

** All sensitivities quoted are for dry air at 22 C and 760 mm Hg

Table 5

Correction factors introduced when measuring ^{60}Co exposure rate with the VNIIM standard device for 0 3 to 3 MeV *

k_1	1 085
k_2	1 074
k_3	1 073
k_4	0 950
k_5	0 992
k_6	0 988
Total correction factor	1 164 ± 0 013

Maximum uncertainty of absolute exposure determination ±1 5 %
*Conditions chamber air pressure 10 atm temperature 20 C chamber field strength 440 V/cm

Robert Roessle Clinic of the DAW The measurements were made at the rotation centre of the unit (SCD 55 cm) to eliminate the distance error through irradiation with the two opposite positions of the source The field size was 15 cm × × 15 cm at a distance of 55 cm, and the exposure rate under these conditions on 1 November 1966 was 55 R/min

The 2 MV roentgen source of the NPL Teddington The NPL uses a roentgen ray beam produced by a 2 MV Van de Graaff generator, having a tungsten transmission target and a focal spot of approximately 1 mm diameter The diaphragm system was described by Aston & Smith (1954) and this produces a canalized beam, circular in cross-section The broad spectrum of radiation can be represented by an equivalent spectrum with only three monoenergetic components (Barnard et coll 1959) More recently (Barnard, Marsh & Hitchman 1964) good experimental agreement was reached with spectra calculated according to Kramers method (1923)

For the measurements described in the paper, an aperture size was chosen so as to give a beam diameter of 4 5 cm at the calibration distance of 180 cm so that the ionization chambers were completely enveloped The HVT of the roentgen beam was 12 mm Cu and the exposure rate at 180 cm was approximately 12 R/min

^{60}Co radiation source of the primary standard of the VNIIM Leningrad The ^{60}Co gamma emitter used for the comparison measurements in Leningrad with an activity of 4 5 Ci on 1 June 1966 (diameter 4 mm, height 4 mm, surrounded by a capsule of stainless steel of 1 mm wall thickness), is placed into a lead container of 35 cm diameter in a special holder The diameter of the emerging beam

meters of 30 and 60 mm (corresponding to field diameters of 120 and 240 mm at a distance of 60 cm). The results of the comparison are presented in Table 7. The considerable scatter of the measured values is presumably due to the inaccuracy in determining the chamber source distance at the time of that comparison. The results showed that the response of the DAW chambers obtained at the two institutes differed by not more than 1 per cent.

Conclusion

The final results of the comparisons between the primary ionization chambers of the three countries involved are summarized in Table 8. Using correction factors thought by the respective centres to be most appropriate, these results show agreement to about 1 per cent. This is within the estimated limits of accuracy claimed by each of the laboratories. It is reasonable to suppose therefore that the realization of the unit of exposure by means of high pressure and cavity ionization chambers is accurate to within about 2 per cent. This provides confirmation of the validity of secondary standard calibrations for other countries carried out by the three centres concerned in the present intercomparisons.

Acknowledgements

The comparison carried out at the NPL was based upon the standards and corrections established principally by Dr G. P. BARNARD to whom the authors would like to dedicate their contribution in memoriam.

SUMMARY

The primary standard cavity ionization chambers of DAW Berlin were compared with the primary standard high pressure ionization chambers of VNIIM Leningrad using their ^{60}Co unit, and with the primary standard cavity ionization chambers of NPL Teddington using their 2 MV Van de Graaff roentgen ray generator. With correction factors thought by the respective centres to be most appropriate, the comparisons showed agreement to about one per cent, which is within the estimated limits of accuracy claimed.

ZUSAMMENFASSUNG

Hohlraumionisationskammern der DAW Berlin wurden mit der Primarstandard Druckluft Ionisationskammer des VNIIM Leningrad an einer ^{60}Co Einheit und mit den Primarstandard Ionisationskammern des NPL Teddington an dessen 2 MV Van de Graaff Generator verglichen. Unter Verwendung der von den entsprechenden Institutionen bestimmten Korrektionsfaktoren zeigen die Vergleich Übereinstimmung auf etwa ein Prozent, was innerhalb der Fehlergrenzen liegt.

Table 8

Summary of international comparisons on DAW Chamber C 1.5 No. 2

Standardising laboratory	NPL		VNIIM	
Measurement or correction by	NPL	DAW	VNIIM	DAW
Mean uncorrected sensitivities $C/R \times 10^{10}$	4.64	4.65	5.31	4.64
Mean corrected sensitivities $C/R \times 10^{10}$	4.54	4.59	4.56	4.60

can be varied by using lead diaphragms of from 30 mm to 60 mm diameter at a distance of 15 cm from the source. Radiation scattered in the container and diaphragm with 30 or 60 mm diameter of the diaphragm is 2 or 4 %, respectively (TUGIN et coll. 1965). The exposure rate at 50 cm distance was about 0.3 R/min on 1 June 1966.

Results of the intercomparisons

Comparisons with the cavity ionization chambers of the DAW and the NPL. Comparative measurements with the cavity ionization chambers of the two institutions were carried out at NPL during September 1965 and March 1967. The same charge measuring apparatus including standard capacitor was used for all chambers and the intercomparison was carried out via a whole beam monitor chamber. All the chambers used in the experiments were unsealed, and, because it was assumed that all the chambers responded equally to changes in *ambient air* conditions, there was no need to apply any corrections for different ambient air conditions in the experiments. The cavity chambers of both institutions were of similar size so that no corrections for lack of beam uniformity were necessary.

From this intercomparison it appears that the ratio of the response of the DAW chamber C 1.5 No. 2 to the mean response of the NPL chambers is unity. For the air equivalent chamber H III/1.5 No. 1 this ratio is 1.01. If the corrections to the chambers as determined by their owners are applied then the exposure as determined by NPL is 1 per cent greater than that determined by the DAW chambers. These results are listed in Table 6.

The deviations in determining exposure are thus attributable to the respective differing corrections that are used.

Comparison between the cavity ionization chambers of DAW and the high pressure chamber of VNIIM. The comparison measurements were made in May 1966 at source chamber distances of 60 cm and 80 cm and for diaphragm dia-

meters of 30 and 60 mm (corresponding to field diameters of 120 and 240 mm at a distance of 60 cm) The results of the comparsion are presented in Table 7 The considerable scatter of the measured values is presumably due to the inaccuracy in determining the chamber source distance at the time of that comparison The results showed that the response of the DAW chambers obtained at the two institutes differed by not more than 1 per cent

Conclusion

The final results of the comparisons between the primary ionization chambers of the three countries involved are summarized in Table 8 Using correction factors thought by the respective centres to be most appropriate these results show agreement to about 1 per cent This is within the estimated limits of accuracy claimed by each of the laboratories It is reasonable to suppose therefore that the realization of the unit of exposure by means of high pressure and cavity ionization chambers is accurate to within about 2 per cent This provides confirmation of the validity of secondary standard calibrations for other countries carried out by the three centres concerned in the present intercomparisons

Acknowledgements

The comparison carried out at the NPL was based upon the standards and corrections established principally by Dr G P Barnard to whom the authors would like to dedicate their contribution in memoriam

SUMMARY

The primary standard cavity ionization chambers of DAW Berlin were compared with the primary standard high pressure ionization chambers of VNIIM Leningrad using their ^{60}Co unit and with the primary standard cavity ionization chambers of NPL Teddington using their 2 MV Van d Graaff roentgen ray generator With correction factors thought by the respective centres to be most appropriate the comparisons showed agreement to about one per cent which is within the estimated limits of accuracy claimed

ZUSAMMENFASSUNG

Hohlraumionisationskammern der DAW Berlin wurden mit der Primarstandard Druckluft Ionisationskammer des VNIIM Leningrad an einer ^{60}Co-Einheit und mit den Primarstandard Ionisationskammern des NPL Teddington an dessen 2 MV Van de Graaff Generator verglich n Unter Verwendung der von den entsprechenden Institutionen bestimmten Korrektionsfaktoren zeigen die Vergleiche Ubereinstimmung auf etwa ein Prozent was innerhalb der Fehlergrenzen liegt.

RÉSUMÉ

Les auteurs ont comparé les chambres d'ionisation à cavité étalons primaires de la DAW de Berlin avec les chambres d'ionisation etalons primaires à haute pression du VNIIM de Léningrad au moyen de leur unité à ^{60}Co et avec les chambres d'ionisation à cavité étalons primaires du NPL de Teddington au moyen de leur générateur de rayon de roentgen de Van de Graaff à 2 MV En tenant compte des facteurs de correction considérés comme les meilleurs par les differents centres ces comparaisons ont montre une concordance a environ un pour cent près ce qui est dans les limites de précision attendue

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PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Dose effect relationship in ^{90}Sr induced bone tumours

by

AGNAR NILSSON

In spite of a very extensive literature concerning the carcinogenic effect of radiostrontium there seem to be only a few reports dealing with the various problems related to dose dependency Studies in this field (FINKEL *et coll* 1957) have mostly been concerned with the relationship between dose and frequency of mice having overt macroscopically detectable osteosarcomas. Many questions still remain which seem to be of interest for a better understanding of the dynamics of tumour formation and its relation to dose and time In a previous report the main attention was focussed on the ^{90}Sr induced carcinomas and their localisation in the mucous membranes of the head their development and relation to dose (NILSSON 1968) A more detailed study concerning the effect of blood and blood forming tissues and their dependency on dose will be presented in a further paper

The purpose of the present work was to obtain more information concerning the dose-dependent histologic changes in bone and their relation to frequency location and type of bone tumours The relation between dose time of appear

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Table 1

Experimental conditions and the ^{90}Sr doses employed

Dose of ^{90}Sr in $\mu Ci/g$ body weight	Total number of mice investigated	Number of mice killed in groups of five	Last day for sacrifice	Number of spontaneously dead mice
1.6	120*	65	300	50
0.8	121	75	360	46
0.4	122	95	480	27
0.2	122**	103***	540	17
Control	95	94****	570	1

Out of these animals five* and two** were lost during the experiment, only three*** and four**** animals, respectively, were sacrificed in the last test group.

ance and type of histologic changes and fluctuations in the bone cell populations was studied, as well as their possible relation to later development of tumours. The influence of the ^{90}Sr dose on tumour growth rate and latency time was also taken into account.

Material and Methods. Four groups of CBA mice, 75 days old, were treated intraperitoneally with $^{90}Sr(NO_3)_2$. A group of 95 animals not treated with ^{90}Sr were used as control. At intervals of 7, 14, 21 and 30 days after injection of ^{90}Sr, and then at monthly intervals, five mice from each were selected at random and sacrificed, until all mice in each series had been utilized. The experimental conditions and the ^{90}Sr doses employed are recorded in Table 1. The animals were examined roentgenologically before being killed and the roentgenograms were used as a guide for locating tumours.

The mice were sacrificed by cervical dislocation. Both femurs, tibias and humerus, the spine, pelvic bones and the head were fixed in Stieve's fluid and decalcified in 20 % formic acid for histologic examination. Conventional methods were used, the sections being stained according to the van Gieson method, haematoxylin eosin, Lillie's azure eosinate, Masson's trichromemethod, PAS according to Hotchkiss and Foot and Foot's silver method. The animals that died spontaneously were autopsied and treated in the same way.

The numbers of osteoblasts and osteoclasts were determined per millimeter endosteum lining the epiphyseal metaphyseal part of the distal femur, at various intervals of time between 7 and 210 days after injection of ^{90}Sr (Fig. 1). The bone sections were investigated in a Wild microscope equipped with a Wild Zeichentubus. The field was projected at a magnification of 1 250 diameters and the endosteum was measured with an odometer. At each interval, 10 fields



Fig 1 Epiphyseal plate and metaphyseal bone of femur 30 days after injection of 0.4 μCi ^{90}Sr/g body weight Osteoblasts and osteoclasts were counted in a zone immediately below the epiphyseal cartilage van Gieson × 125

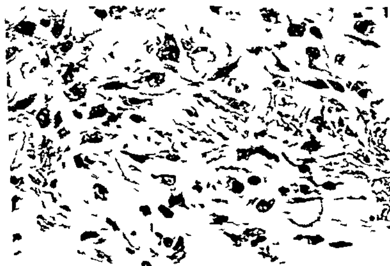


Fig Non neoplastic fibroblastic proliferation in bone marrow femur 210 days after injection of 0.8 μCi ^{90}Sr/g body weight van Gieson × 500



Fig 3 Non neoplastic focal proliferation of osteoblast like cells and osteoclastic activity along the endosteal linings in diaphysis tibia 218 days after injection of 0 4 μCi ^{90}Sr/g body weight van Gieson $\times$ 500



Fig 4 Solitary neoplastic bud inside non neoplastic fibroblastic tissue from lumbar vertebra 300 days after injection of 0 8 μCi ^{90}Sr/g body weight van

Table 2

Cause of spontaneous death in relation to doses of ⁹⁰Sr administered per gram body weight

	1 6 µCi		0 8 µCi		0 4 µC		0 2 µCi	
	No of mice	Mean survival days	No of mice	Mean survival days	No of mice	Mean survival days	No of mice	Mean survival days
Acute aplastic anaemia	6	18 2						
Chronic anaemia	9	263	5	226			1	358
Osteosarcoma	19	259 9	30	319 5	8	408 9		
Squamous cell carcinoma	16	282 5	3	338 3				
Leukaemia			3	250	11	264 5	4	251 3
Adenocarcinoma							1	402
Angiosarcoma			1	314			1	425
Fibrosarcoma							1	347
Liver rupture (hepatoma)							1	422
Liver rupture (carcinoma of the liver)							1	439
Not settled			4	350 0	8	293 8	7	383 4
Total	50	238 7	46	312 5	27	316 0	17	357 8

were counted in each of five femurs The number of cells per millimeter of endosteum was determined by dividing the aggregate number of cells counted by the total length of the endosteum measured for the fifty fields The cells supposed to be osteoblasts were characteristically arranged in a one cell thick layer carpeting the bone surfaces. The cells were either columnar in shape or they were thin flattened or later on fusiform elements The osteoclasts were large multi- or uni nucleated cells present on or near bone and in process of absorption

The number of nucleated chondrocytes in the epiphyseal cartilage were determined at the same time intervals as the mean value of 10 unit areas of 6 400 μ^2 from both femurs of five mice Chondrocytes consisted of single or multiple cells residing in lacunae in the intercellular substance of cartilage

The mice were fed during the experiment on a standard diet ad libitum and kept in the same room under similar environmental conditions

Histologic evaluation

The histologic events leading to overt paraosteal tumour growth in this material were defined as follows

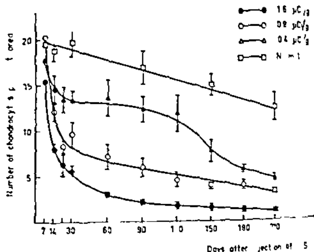


Fig 5 Number of chondrocytes per unit area (6 400 μ^2) related to dose and time after injection of ^{90}Sr Confidence limit 67 %

Non neoplastic proliferation Distinct proliferation of uniform reticular cells with a histologic appearance of fibrosis (Fig 2), with or without formation of foci of endosteal cell elements (Fig 3), in both cases without neoplastic characteristics

Osteosarcoma buds (microscopic tumours) Completely intramedullarily situated foci of proliferations containing pleomorphic cell elements (Fig 4) Buds of this type have earlier been shown to be transplantable and to have malignant characteristics (NILSSON 1962)

Overt macroscopic tumours Tumours breaking through the compact bone with or without infiltrating the surrounding soft tissue

Results

A survey of the pathologic findings among spontaneously dead animals in the various dose groups are listed in Table 2 Some mice in the 1 6 µCi group died early due to haematopoietic disorders Of more interest is however that the animals succumbed from carcinomas nearly as often as from osteosarcomas The cause of death in the 0 8 µCi group was predominantly osteosarcomas, and in the 0 4, and possibly also in the 0 2 µCi group it was leukaemia It should however be observed that many of the mice who died from carcinomas had osteosarcomas or leukaemia as well

Pathologic features of the skeleton

Histologic appearances in relation to dose and time The variations in the distal f... in histologic appearances in relation to dose were distinguished by an

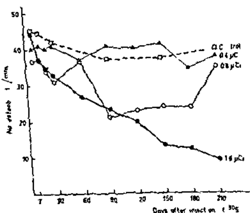


Fig 6 Number of osteoblasts per millimeter endostium of distal metaphysis of femur related to dose and time There was a significant difference between the 0 8 µCi and 1 6 µCi groups at 7 days (t = 2 39? p < 0 025) 60 days (t = 3 560 p < 0 005) and 150–210 days (t varied between 4 310 and 6 471 p < 0 001) The 0 4 µCi group differed significantly from the 0 8 µCi group at 21 days (t = 2 352 p < 0 01) 30 days (t = 3 421 p < 0 001) 90—150 days (t varied between 4 585 and 6 670 p < 0 001) and 180 days (t = 2 ?04 p < 0 05) after injection of ⁹⁰Sr

earlier start and greater severity with increasing dose The chondrocyte columns in the epiphyseal plate were disorganised and hypertrophic chondrocytes occurred associated with a broadening of the plate The dose effect on the number of nucleated cartilage cells in the epiphyseal plate is demonstrated in Fig 5 The epiphyseal cartilage was strongly devitalized in the 1 6 µCi group already after 90 days In the 0 4 µCi group on the other hand a tendency to regeneration was observed after 30—120 days

It is hardly possible to draw up a general schedule for the early and intermediate changes in the osteoblast and osteoclast populations Quantitatively there was a depletion of the osteoblast population the size and duration of which was related to dose (Fig 6) Quantitatively the osteoblasts were transformed into a more fusiform type of cells more rapidly with increasing dose Such cells appeared in the 1 6 µCi group after 7 days but not until about 60 150 and 360 days in the respective 0 8 0 4 and 0 2 µCi groups

The number of osteoclasts increased in all dose groups during the first few months after ⁹⁰Sr injection (Fig 7) The osteoclasts during the initial phase were localized mostly inside resorption cavities Most of these cavities had lost their osteoclasts after 60 –120 days Instead particularly after 150 days in the 1 6 µCi group immature bone occurred in these cavities and along endosteal linings this was a basophilic PAS positive coarse fibred bone containing only a few fusiform atypical osteoblast elements (Fig 8) It is notable that these changes in the distal femur of the 1 6 µCi group seem to constitute a cessation of the cellular activity as apparent from the lack of focal proliferations Apposition of immature bone was seen to a much lesser extent in the 0 8 µCi group and in the other two groups it was quite insignificant Synchronously with an increasing

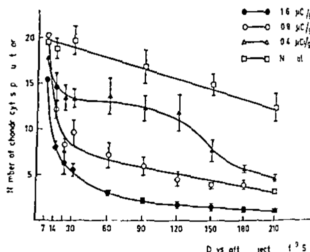


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Pathologic features of the skeleton

Histologic appearances in relation to dose and time The variations in the distal femur in histologic appearances in relation to dose were distinguished by an



Fig 8 Heavy destruction of epiphyseal plate and metaphyseal bone apposition of basophilic immature coarse fibred bone 270 days after injection of 1 6 μCi ^{90}Sr/g body weight van Gieson × 300

show the lowest incidence of these changes in the femur the 0 8 μCi group the highest and the 0 4 group a frequency about four times as great as the 1 6 group

Intramedullary tumour buds The further development of the non neoplastic proliferations into intramedullary tumours consists of a successive confluence of expanding multicentric centres of proliferating osteoblast like cell elements These cells attain in time a more atypical appearance lay down immature bone and acquire the histologic characteristics of an osteoblastic osteosarcoma During neoplastic transformation of the fibroblastic type there seems to be constant formation of small solitary or multiple foci or accumulations of tightly packed atypical cells inside the non neoplastic fibrous tissue (Fig 4) The induction time for intramedullary osteosarcomas was related to dose Generally the osteoblastic type appeared earlier than the fibroblastic buds On the other hand the mean induction time for the two tumour types did not differ significantly

Rate of transformation For fibroblastic osteosarcomas there existed a dose related influence on the rate of transformation of non neoplastic proliferations into intramedullary osteosarcoma buds and further into overt paraosteal tumours The rate of development was significantly increased in the 1 6 μCi group as compared with the other series No such relation was found for the osteoblastic type Table 3)

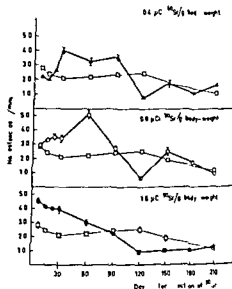


Fig 7 Number of osteoclasts per millimeter of endostium in the distal metaphysis of femur related to dose and time in the controls

activity of the osteoblast population, endosteal foci of proliferating osteoblast like elements appeared Such foci were seen in the 0 8 μCi group after 180—210 days but not until 300 and around 500 days, respectively, in the 0 4 and 0 2 μCi groups These types of changes were not so striking in the 0 4 μCi group, instead, increased activity and proliferation of reticular cells predominated

The histologic changes described were also studied in other bones The destructive changes inside each group were generally of about the same magnitude in the tibia and femur but usually of much less severity in other bones, particularly in thoracic and cervical vertebrae

Tumour development

Non neoplastic proliferations The occurrence of scattered, focally situated, proliferations of osteoblastic cell elements in resorption cavities or along endosteal linings (Fig 8) as well as a diffuse increase of reticular cells (Fig 2) could be detected in all the different dose groups The relationship between dose and the first appearance and the rate of transformation of these changes into tumour entities was studied more thoroughly for the fibroblastic types of tumour since the different stages of their development are more clearly defined and easier to follow than the osteoblastic types (SUNDELIN & NILSSON 1968) The frequency and start of the changes were dependent on dose and time after injection of ^{90}Sr (Fig 9) In the 1 6 μCi group these changes were usually most numerous in the lumbar vertebrae and pelvic bones In the other groups the long bones, particularly the femur, were most often involved The 0 2 and 1 6 μCi groups

Table 3

Influence of dosa e on rate of tumour de'elopment cal ulated from cur'es in Fig 8 by hori ontal retrograde extrapolation from the points representin respectively detected osteosarcoma buds to the curves for detected non neoplastic proliferations and from macroscopic tumours to the curves for detected tumour buds

Dose	Tumour	Horizontal retrograde extrapolation of time interval between	
		Detected tumour buds and curve represent ing non neoplast c p ol ferations days ± SE	Detected macroscopic tumours and curve rep resenting tumour buds days ± SE
1 6 μCi		21 3±4 3 (n 19)	29 2±1 6 (n 5)
0 8	Fibroblast c	58 1±0 9 (n 66)	57 2±2 4 (n 20)
0 4		66 1±3 9 (n 50)	63 1±8 8 (n 8)
1 6 μCi			53 3±2 7 (n 30)
0 8	Osteoblast c		53 6±1 9 (n 54)
0 4			63 ±17 (n 6)
1 6 μCi versus 0 8 μCi		t = 8 364 ($p < 0.0005$)	9 722 ($p < 0.0005$)
	Fibroblastic		
0 8 μCi versus 0 4 μCi		t = 1 995 ($p < 0.025$)	0 648 (not significant)

Tumour bearing animals tumour incidence and tumour multiplicity The highest frequency of tumour bearing mice amongst both spontaneously dead (89 1 %) and sacrificed animals (76 7 %) was found in the 0 8 μCi group (see Table 4) More than half the number of mice in this group had macroscopic tumours while considerably fewer macroscopic tumours were seen in the other groups

The total number of tumours in the various groups can be directly compared since the total number of mice was the same in each group (Table 4 Fig 10) A total of 609 tumours were found in the whole material Of this total number 35 9 % 47 9 % 14 8 % and 1.3 % were located in the groups which started with the highest dose Of these tumours 37 (17 1 %) in the 1 6 μCi group and 79 (27 1 %) 17 (18 9 %) and 1 (12.5 %) in the lower dose series respectively were of macroscopic size For all but the lowest dose group a considerable tend ency towards occurrence of multiple tumours was observed (see Table 5)

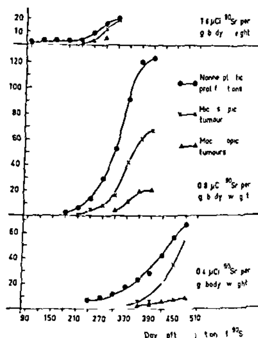


Fig 9 Accumulated number of fibroblastic non neoplastic proliferations microscopically intra medullary and macroscopically extramedullary fibroblastic osteosarcomas

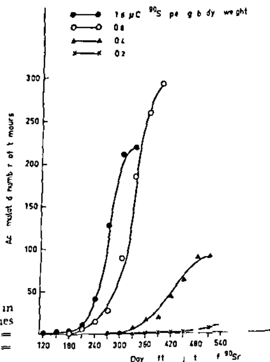


Fig 10 Total number of tumours occurring in the different dose groups The latency times were 1 6 μCi group=267 6±4 6 0 8 μCi= 320 7±5 4 0 4 μCi=426 7±9 8 0 2 μCi= 485 1±37 8

Table 4 *(cont)*

0 8 μCi			0 4 μCi			0 2 μCi		
Total number of mice	Number of mice with tumour	Total number of tumour	Total number of mice	Number of mice with tumour	Total numbar of tumours	Total number of mice	Number of mice with tumour	Total number of tumours
46	41 (83 1 %)	195	97	11 (40 7)	20	17	1 (5 9)	1
45	0	0						
5	[illegible]	5						
5	4	8	55	0	0			
5	3	7	5	1	1	60	0	0
5	3	12	5	0	0	5	1	1
5	4	20	5	3	6	5	0	0
5	5	40	5	2	3	5	0	0
			5	1	2	5	0	0
			5	5	20	5	0	0
			5	4	12	5	0	0
			5	5	26	5	0	0
						5	2	4
						3	2	2
7	3 (30 7)	97	95	21 (22 1)	70	103	5 (4 9)	8
	76 7			52 5			10 4	

increasing dose for tumour development also in the thoracic spine and the head Inside the particular long bones there was a location pattern which was obviously related to dose Most tumours in the 1 6 μCi group were located in the proximal ends of the femur tibia and humerus The same applied in the 0 8 μCi series as regard tibia and humerus but in the femur the distal metaphysis was the pre dominating site In the 0 4 μCi group on the other hand the diaphysis of these bones was most often involved (Table 7)

The location of tumours in relation to the type of osteosarcoma differed with respect to their sites in the different parts of the skeleton In all the dose

Table 4

Tumour frequency and total number of tumours in relation to dose of ^{90}Sr in μCi/g body weight and time

Spontaneously dead mice	1.6 μCi		
	Total number of mice	Number of mice with tumour	Total number of tumours
	50	42 (84.0 %)	137
Sacrificed mice			
Day 7—90	30	0	0
120	5	1	1
150	5	1	1
180	5	1	1
210	5	3	5
240	5	5	22
270	5	5	28
300	5	5	29
330			
360			
390			
420			
450			
480			
510			
540			
Total sacrificed mice	65	21 (32.3 %)	87
Per cent of sacrificed mice with tumours after appearance of first tumour		60.0	

Induction time. As may be seen from Fig. 10 the latency period was considerably longer with decreasing dose, though it may be difficult from an experiment of this type to calculate the true mean induction with certainty.

Tumour location. A definite correlation existed between dose and tumour location (Table 6). In the 0.8 μCi group and the 0.2 μCi group more than 50 % of the tumours were located preferentially in the long bones, particularly the femur. In contrast, most tumours in the 1.6 μCi group were sited in the lumbar, sacral spine and pelvic bones (56 %). There was a preference with

Table 4 *(cont)*

0 8 μCi			0 4 μCi			0 2 μC		
Total number of mice	Number of mice with tumour	Total number of tumours	Total number of mice	Number of mice with tumour	Total numbar of tumours	Total number of mice	Number of mice with tumour	Total number of tumours
46	41 (89 1)	195	27	11 (40 7)	20	17	1 (5 9 °)	1
45	0	0						
5		5						
5	4	8	55	0	0			
5	3	7	5	1	1	60	0	0
5	5	17	5	0	0	5	1	1
5	4	20	5	3	6	5	0	0
5	5	40	5	2	3	5	0	0
			5	1	2	5	0	0
			5	5	20	5	0	0
			5	4	12	5	0	0
			5	5	26	5	0	0
						5	2	4
						3	2	2
75	23 (30 7)	97	95	21 (22 1)	70	103	5 (4 9)	8
	76 7			52 5			10 4	

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The location of tumours in relation to the type of osteosarcoma differed with re pect to their sites in the different parts of the skeleton In all the dose

Table 5

Tumour multicentricity in relation to dose

Dose group µCi/g body weight	Number of osteosarcomas/mouse		
	Spontaneously dead	Sacrificed after appearance of first tumour	Whole experiment
1 6	2 6	2 5	2 6
0 8	4 2	3 2	3 8
0 4	0 74	1 8	1 3
0 2	0 06	0 16	0 13

Table 6

Anatomical distribution of osteosarcomas in relation to dose delivered in µCi/g body weight

Location	Per cent tumours in each dose group				Total number of tumours in each dose group			
	1 6 µCi	0 8 µCi	0 4 µCi	0 2 µCi	1 6 µCi	0 8 µCi	0 4 µCi	0 2 µCi
Femur	8 2	28 8	28 9	(25)	18	84	26	2
Tibia	5 9	13 4	14 4	(37 5)	13	39	13	3
Humerus	5 9	12 7	11 1	(12 5)	13	37	10	1
Total long bones	20 1	54 8	54 4	(75 0)	44	160	49	6
Pelvic bones	22 8	13 7	17 8		50	40	16	
Cervical spine	3 7	3 1	2 2		8	9	2	
Thoracic spine	6 8	2 1			15	6		
Lumbar spine	22 8	11 0	5 6		50	32	5	2
Sacral spine	10 5	5 5	11 1	(25 0)	23	16	10	
Lumbar and sacral spine	1 4	0 3			3	1		
Coccygeal spine	2 7				6			
Total vertebral column	47 9	21 9	18 9	(25 0)	105	64	17	2
Head	7 8	4 5	2 2		17	13	2	
Multiple site	1 4	5 1	6 7		3	15	6	
Total					219	292	90	8

Table 7

Distribution of tumours in various parts of the long bones in relation to the total number of tumours in each of the long bones within the dose group

μCi Sr/g body weight	Total number of tumours	Distribution of tumours in					
		Distal epiphysis		Diaphysis		Proximal epiphysis	
		Number	Per cent	Number	Per cent	Number	Per cent
Femur							
1 6	18	1	5 6	4	22 2	11	61 1
0 8	81**	38	45 2	22	26 2	17	20 ?
0 4	26	4	15 4	15	57 7	4	15 4
Tibia							
1 6	13	2	15 4	7	53 8	4	30 8
0 8	39	1	2 6	5	12 8	32	82 1
0 4	13*	0	0 0	8	61.5	3	23 1
Humerus							
1 6	13	3	23 1	1	7 7	9	69 2
0 8	37*	4	10 8	6	16 2	25	67 6
0 4	10	0	0 0	9	100 0	0	0 0

The number of tumours that could not be located to a particular zone are indicated as follows one tumour ( ) two tumours (*) three tumours ( ) seven tumours ( )

groups the humerus and the lumbar and the sacral spine had the highest frequency of fibroblastic osteosarcoma In the pelvic bones there was a strong predominance of osteoblastic osteosarcoma in the 1 6 μCi group whereas in the 0 4 μCi group it was of fibroblastic type All tumours in the head were of the osteoblastic type As regards the site inside particular bones the fibroblastic intramedullary osteosarcoma buds in the 0 4 μCi group occurred to a much greater extent in the diaphyseal part of the long bones than did the osteoblastic tumour buds

Tumour types With decreasing dose there was a shift from a prodominance of osteoblastic to fibroblastic osteosarcomas (Table 8)

Discussion

Relationship between dose and tumour frequency in various tissues Each of the different ⁹⁰Sr doses administered in this investigation proved capable of inducing osteoarcomas In previous studies (FINKEL 1958) the same was reported with

Table 8

Histological classification of bone tumours

Type of tumours	Percentage distribution by dose			
	1.6 μCi n = 219	0.8 μCi n = 292	0.4 μCi n = 90	0.2 μCi n = 8
Osteoblastic osteosarcomas	87.2	68.8	24.4	(50.0)
Fibroblastic osteosarcomas	11.0	29.8	71.1	(37.5)
Chondroblastic osteosarcomas	0.9	—	—	
Angiosarcomas	0.9	2.1	4.4	(12.5)

doses even lower than we used. The optimal dose, 0.8 μCi/g body weight, was in good agreement with earlier reports (FINKEL 1958, NILSSON 1962). The frequency of osteosarcomas was still very high in the 1.6 μCi series and, in addition, carcinomas occurred in the mucous membranes of the mouth and nose, as reported in a separate paper (NILSSON 1968). In the highest dose group, leukaemia was not detected among the spontaneously dead mice. This was the case in each of the other series, however, the highest frequency being in the 0.4 μCi group. The existence of a clear optimum of carcinomas, osteosarcomas and leukaemia for the doses 1.6, 0.8 and 0.4 μCi/g, respectively, seems to indicate a strong dependency on dose and on the topographic location of the tissue involved. The radiation delivered from a certain dose of radiostrontium to the mucous membranes close to bone is much less than to the osseous tissues and to the bone marrow. Thus, doses inducing carcinoma may generally be too destructive for induction of osteosarcomas at a maximum rate, and even more so for induction of leukaemia. Only doses with an effect not more destructive than allowing recovery and a certain degree of regeneration will promote an optimal frequency of neoplasia. It was also shown in an earlier investigation (NILSSON 1967) that female mice injected with an optimal osteosarcoma dose had a significantly reduced number of osteosarcomas when an increased excretion (30 %) of ^{90}Sr was brought about by lactation. At the same time, however, there was a significant increase of leukaemia compared with non-lactating mice.

Relation between dose and latency time. As previously demonstrated in this investigation, there was a correlation between dose and latency time not only for the first occurrence of neoplastic changes but also for the appearance of the destructive and regenerative phases and non-neoplastic proliferations (Figs. 9 and 10). This clearly indicates that the latency time is a function of dose and time

In this relation the total accumulated dose seems to be a more decisive factor in tumour genesis than the initial dose rate (NILSSON et coll 1967, VAN PUTTEN 1961)

Rate of tumour development After becoming established as a stage of development the fibroblastic non neoplastic proliferations were more rapidly transformed into intramedullary fibroblastic osteosarcomas and to overt neoplasms within the higher dose groups (Table 4) There was thus a significantly more rapid development of the fibroblastic tumours in the 1 6 than in the 0 8 and 0 4 µCi groups The reason for this might be a combination of factors, such as the occurrence of more selectively altered, radioresistant cell clones after higher doses the growth of which may not be suppressed since the immunologic capacity and resistance of the host are diminished

The growth rate of osteoblastic buds was not enhanced by dose (Table 4) These tumour buds from the histologic point of view are more highly differentiated than the fibroblastic ones and consequently might not be influenced in the same degree by irradiation The reason for the general reduction of the mean induction time with increasing dose seems to be primarily the earlier occurrence of tumour cell clones and not a generally increasing growth rate

Relation between dose and tumour multiplicity It is seen from Table 5 that the multicentric genesis of tumours is a function of time and dose since with increasing survival there is a perpetual rise in the number of tumours in each group of animals

Considering the relation between tumours of macroscopic and microscopic extent it is of interest to note that the latter predominated strongly in the 0 8 µCi group Many microscopic tumours *develop with a* superoptimal not too destructive dose but the survival time for the mice is too short for their transition into macroscopic entities The induction time with a suboptimal dose might be too long for the development of an appreciable number of macroscopic tumours

Dose related histologic variations in bone tissue When comparing homologous bones between different dose groups, the degree and extent of histologic changes have been found to be strongly dose-dependent Such differences are however also obvious when comparing heterologous bones inside each group This variation seem to be related to the fact that the radiation dose absorbed is determined by many factors such as the size geometry and metabolic rate of the bones This may explain the more serious effect on the femur than on the lumbar vertebrae and why the latter are more damaged than the thoracic vertebrae

In order to transform the various radiation doses into terms of their mutual biological effect the nucleated chondrocytes in the epiphyseal plate of the distal

femur were counted (Fig 5) These cells were expected to provide a better reference than the osteoblasts to the dose relations in this part of the bone Only in the 0 4 μCi group were there signs of recovery and a temporary regeneration In the two other groups, and particularly in the 1 6 μCi group, there was a continuously enhanced cellular depletion with increasing dose The osteoblastic and osteoclastic populations in the distal femur showed more variations (Figs 6 and 7) There was thus a continuously decreasing number of cells in the 1 6 μCi group In the group given 0 8 μCi, which is the optimal osteosarcoma dose, the osteoblastic population was forced into a state of instability characterized by a diphasic fluctuation The recovery observed after 60 days might be characterized as an abortive regeneration, which may indicate its origine from a cell population so heavily damaged that it could only survive a limited number of divisions The second regeneration which started after about 180 days appeared to give birth to scattered, focal, non neoplastic proliferations (Figs 2 and 3) which, however, successively were transformed into tumours This might indicate that cells originating from this second regeneration were selected, possibly quite radioresistant, but defective, and potentially malignant cell elements The destructive effect of the highest dose will not allow any appreciable degree of regeneration in the distal femur, as mirrored by the low incidence of tumours in the latter The 0 4 μCi dose, on the other hand, may not cause damage sufficient to induce the instability necessary in the osteoblastic population to create optimal conditions for tumour development Of particular interest is however that the changes most prominent in the 0 4 μCi group were located in the reticular cells of the bone marrow to a much higher degree than in the other groups These cells give rise predominantly to the fibroblastic type of osteosarcomas (Fig 4) Some observations made in the 0 4 μCi group as well have shown advanced destruction and necrosis in the bone marrow, including the reticular cells This might be an expression of circulatory disturbances inside the bone marrow A lesser radioresistance of the reticular cells compared with that of the osteoblastic elements might also explain why the osteoblastic elements more often proliferate in the higher dose groups

Relation between dose and tumour location The importance of dose for the tumour location is illustrated in Table 7 There is a remarkable shift from a significant predominance of tumours in the long bones particularly the femur, in the 0 8 and the 0 4 μCi groups to a clear under representation in the 1 6 μCi series The number of tumours in the latter group is thus only 28 % of that in the 0 8 group In the pelvic bones and the spine there are instead 20 % and 39 % more tumours in the 1 6 μCi than in the 0 8 μCi group It should also be pointed out that with increasing dose there are more tumours located in such

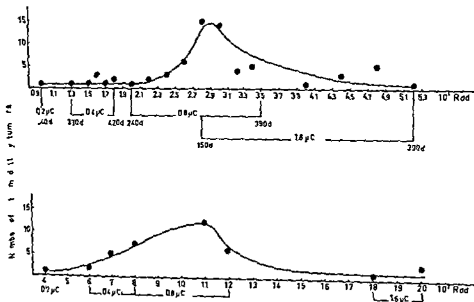


Fig 11 Occurrence of intramedullary osteosarcomas in relation to the total accumulated dose in the different dose groups *Upper curve* dose to the distal and proximal epiphyseal parts of the femur *Lower curve* dose to the midshaft of the femur

small bones as the thoracic and coccygeal vertebrae The same applies to the location of tumours in the head This may be connected with the previously mentioned differences in the radiation doses absorbed in various parts of the skeleton A comparison of the 0 4 and 0 8 μCi groups reveals a fairly constant pattern of tumour location

The tumour sites inside particular bones also reveal a changed pattern with decreasing dose About 25 % of the tumors in the long bones of the 1 6 and 0 8 μCi groups were located in the diaphysis In the 0 4 μCi series 73 % were located there

Relation between total accumulated dose in various bones and tumour frequency It seemed of interest to investigate the influence of the total accumulated dose upon the tumour rate in the various dose groups The femur was chosen as model for this purpose The doses absorbed in the distal epiphysis the diaphysis and the proximal epiphysis of the femur were calculated The mice 75 days old when injected and weighing 21 to 22 g were given 0 8 μCi ^{90}Sr g body weight and the weight of the femur and its various parts were determined According to the investigation of PARMLEY et coll (1962) the absorbed energy

in the whole femur was 38 %. The mean energy of $^{90}Sr-^{90}Y$ is 1.13 MeV per disintegration. On the basis of these data and the retention curves for the femur, an approximative radiation dose to the various parts of the femur can be calculated. The doses in Fig. 11 are given merely as reference values and refer only to microscopic intramedullarily situated osteosarcomas. The doses in Fig. 11 (upper curve) relate to osteosarcoma buds located only in the proximal and distal parts of the femur, and those in Fig. 11 (lower curve) only to buds located in the central part of the diaphysis. There is a range between 9 500 and 52 000 rad, particularly between 27 000 and 31 000 rad, in which most tumours occur. This optimum can be assigned almost solely to the 0.8 μCi group, the dose in the 1.6 μCi group being too high for an optimal tumour induction in the femur.

In the diaphysis of the femur most tumours appeared around 10 000 to 12 000 rad. A possible explanation of this might be that the magnitude of the dose that induces irreversible changes leading to tumour formation is in fact much smaller than indicated by these figures and the figures for the distal and proximal parts. Tumours have exceptionally been observed after only 4 500 rad, and around 9 000 rad in the distal part. On the assumption that a minimum time, and thus a minimum accumulated dose, is necessary for induction of histologic changes successively leading to formation of tumours it seems probable that the dose delivered after this time does not have much importance. The dose is constantly increasing during the time it takes for a tumour to develop. This surplus dose will be higher at certain sites like the distal metaphysis of the femur, since the dose rate there is much higher than in the diaphysis. In some cases this surplus seems to have some effect in promoting the rate of development of fibroblastic osteosarcoma, at least in the highest dose group. No such effect was observed for the osteoblastic type, however. On the other hand, it must be borne in mind that a supraoptimal dose might have a depressing effect initially on tumour induction and later also on its further development.

It may be of interest to note that the frequency of diaphyseal tumours in relation to the total number in the femur was 36 %. The dose delivered was 35 % of that in the distal and proximal epiphysis. As mentioned earlier, most tumours in the 0.8 μCi group occurred in the femur and in the 1.6 μCi group in the spine, particularly the lumbar vertebrae. It would have been of interest to compare the doses delivered to these bones but no such measurements have however been made. A rough approximation from retention curves of femur and lumbar vertebrae, and also from calculations made by MARSHALL & FINKEL (1959), reveals an estimated dose to the lumbar vertebrae of about 50 % of that delivered to the femur. This seems to be the explanation of the optimal tumour frequency in the spine for the highest dose level.

Tumour types. The percentage of fibroblastic tumours was enhanced with decreasing dose. This observation is however contradictory to that of STRELTSOVA (1959) who reported an increasing number of osteoblastic (osteosclerotic) tumours with decreasing dose in rats. In the 0.4 μCi series this type of tumour had a tendency to be overrepresented in the diaphysis of the long bones (69 %), where the radiation dose was only about 30 % of that delivered to the metaphyseal parts of the bone. The mean tumour induction time on the other hand was about 160 days longer than in the 1.6 μCi group.

Some cases of angiosarcoma have been found in all the dose groups but only one chondroblastic osteosarcoma was detected in the 1.6 μCi group. The percentage distribution of angiosarcomas indicates a tendency to increase with decreasing dose.

SUMMARY

The dose dependency was investigated in four groups of male CBA mice injected intraperitoneally with respectively 1.6, 0.8, 0.4 and 0.2 μCi of ^{90}Sr/g bodyweight. A group of 95 animals was used as control. Five mice from each group were sacrificed at certain intervals. It was shown pathologically and by conventional histologic method that not only latency time and tumour frequency but also type, multicentricity, location and to some extent the growth rate of osteosarcomas were related to the dose. The pathologic changes preceding tumour development and their relation to the dose given are discussed.

ZUSAMMENFASSUNG

Die Dosisabhängigkeit wurde in vier Gruppen von männlichen CBA-Mäusen, die intraperitoneal mit bzw. 1.6 μCi, 0.8 μCi, 0.4 μCi und 0.2 μCi von ^{90}Sr/g Körpergewicht injiziert wurden, studiert. Eine Gruppe von 95 Tieren wurde als Kontrolle benutzt. Fünf Tiere aus jeder Gruppe wurden bei verschiedenen Intervallen getötet. Pathologisch und histologisch wurde gezeigt, dass nicht nur die Latenzzeit und Frequenz der Tumoren, sondern auch Art, Multizentrizität, Lage und im gewissen Masse Zuwachsrate von Osteosarcomen von der Dosis abhängig sind. Pathologische Veränderungen bevor Beginn der Tumorentwicklung im Verhältnis zur gegebenen Dosis werden diskutiert.

RÉSUMÉ

L'auteur a étudié l'influence de la dose sur quatre groupes de souris mâles CBA auxquelles était injecté par voie intrapéritonéale respectivement 1.6 μCi, 0.8 μCi, 0.4 μCi et 0.2 μCi de ^{90}Sr/g de poids corporel; un groupe de 95 animaux a servi de témoin. Cinq souris de chaque groupe ont été sacrifiées à certains intervalles. Les méthodes anatomo-pathologiques et histologiques habituelles ont montré que non seulement le temps de latence et la fréquence des tumeurs, mais aussi leur type, leur caractère multicentrique, leur siège et dans une certaine mesure la vitesse de croissance des ostéosarcomes sont liés à la dose. Les modifications anatomo-pathologiques qui précèdent le début du développement de la tumeur et leur relation avec la dose administrée sont étudiées.

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RADIOSENSITIVITY OF MEDIASTINAL LYMPHOMAS IN HODGKIN'S DISEASE TREATED WITH SPLIT COURSE RADIOTHERAPY

A retrospective study

by

TORSTEN LANDBERG and HANS FORSLO

Radiotherapy has been the method of choice *in the treatment* of clinically local Hodgkin's disease since 1902 when PUSEY reported good results in malignant lymphomas. Several chemotherapeutics are *now available* but appear to be reserved mainly for patients in whom radiotherapy cannot be expected to produce the desired *effect especially if* the disease is advanced. The belief that Hodgkin's disease is in the end always fatal (PATERSON & PATERSON *1954*) now seems to be giving way to the feeling that it is at least sometimes unicentric in origin and possibly curable by radiation treatment (KAPLAN *1952 1966* EASSON & RUSSEL 1963 JELLIFFE 1965 and EASSON 1966) justifying irradiation of diseased tissues to adequate dose levels.

Opinions differ concerning the dose that should be given in the treatment of local Hodgkin's disease (*Table 1*). PITCOCK et coll (1959) and FAYOS et coll (1963) have reported longer survivals among patients treated with larger doses

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and it has been demonstrated (JELLIFFE & THOMSON 1955, NOETZLI & SHELINE 1962, JELLIFFE 1965 and WESTLING 1965) that the number of recurrences after irradiation diminishes with increasing dose. KAPLAN (1966) presented a curve for different rates of recurrences at various dose intervals, based upon data from the literature which indicated that a dose of 4 000 rad over 4 weeks should be considered the tumoricidal dose in Hodgkin's disease. Time versus dose diagrams for irradiated patients with Hodgkin's disease without and with local recurrences have been presented by SCOTT (1961), SCOTT & BRIZEL (1964), JELLIFFE (1965), FRIEDMAN et coll. (1967) and SEYDEL et coll. (1967). SCOTT (1961) gave a time-dose line that passed through 750 R in 0.45 days and 2 300 R in 20 days, the doses expressed representing the minimal tumor dose as estimated by the prescribing physician. SCOTT & BRIZEL (1964) compared orthovoltage and supervoltage techniques. The doses had been calculated as minimal tumor dose in tissue roentgens along the central axis of the beam. The patients received five treatments a week. The authors stated that *successful treatment with the supervoltage technique required* a significantly larger dose than did the orthovoltage technique. They also reported that the dose required for short treatment times is higher than might be expected. According to JELLIFFE (1965) considerable variations in the radiosensitivity of Hodgkin's disease exist, a view shared by FRIEDMAN et coll. (1967) who also thought that in one and the same patient the radiosensitivity of one lymph node group might vary from another one. Megavoltage radiotherapy required a larger dose than the orthovoltage technique. They recommended 3 500 rad over 4 weeks and gave the dose as 'a tumor dose' but failed to add a precise definition of the term. SEYDEL et coll. (1967) recommended a dose of 3 500 to 4 000 rad also over 4 weeks.

Special attention has been paid in certain reports to the irradiation of mediastinal lymphomas in Hodgkin's disease. HOHL et coll. (1951) pointed out that successful irradiation of the mediastinum often needs a *longer period of* treatment and thus a larger dose. They recommended target doses of between 3 000 and 4 000 rad. NICE & STENSTROM (1955) found that mediastinal involvement in Hodgkin's disease did not worsen the prognosis more than did involvement of any peripheral lymph node group. These authors recommended a minimum of 2 000 tissue roentgens to the tumour over 14 days. LEVITT (1959) recommended 2 500 R over 14 days. NOETZLI & SHELINE (1962) studied 11 recurrences in 22 patients treated for mediastinal Hodgkin's disease. In nine of these, the tissue doses had been 2 000 R or less and the treatment period, 3 months or less. VAETH (1962) investigated 45 cases of recurrent mediastinal Hodgkin's disease and suggested a target dose of 3 500 R but gave no treatment period. FULLER et coll. (1967) recommended 3 500 to 4 000 rad over 4 weeks.

Table 1

Dose/time data recommended for treatment in Hodgkin's disease

Author (s)	Dose/time data
Houl et coll (1951)	3 000—4 000 rad/?
Nice & Stenstrom (1954 1955)	2 000 tissue roentgens/3 weeks
Healy et coll (1955)	1 500—2 500—4 000 R/1 to 3 weeks
Jelliffe & Thomson (1955)	3 500 R/3 5 to 4 weeks
Elkin s (1956)	3 000—3 500 R/2 weeks
Peters & Middlemiss (1958)	2 500 —3 000 R/? weeks
Levitt (1959)	2 500 R/2 weeks
Vaeth (1962)	3 500 R/?
Arthachinta & Ogden (1962)	2 000—2 800 roentgens/2 to 3 weeks
Crosbie (1962)	3 500—4 000 R/3 to 6 weeks
Kaplan (1962 1966)	3 500—4 000 rad/3 to 4 weeks
Lasson & Russel (1963)	2 500—2 750 rad/3 weeks
Salzman et coll (1964)	2 400—3 500 R/3 weeks
Aisenberg (1964)	3 500 R/3 5 to 4 weeks
Jelliffe (1965)	2 500—4 000 rad/2 to 4 weeks
Newall (1965)	2 000—2 500 rad/2 weeks
Fasson (1966)	3 000—3 250 rad/3 weeks
Fuller (1967)	4 000 rad/4 weeks
Fuller et coll (1967)	3 500—4 000 rad/4 weeks
Friedman et coll (1967)	3 500 rad/4 weeks
Synder et coll (1967)	3 500—4 000 rad/4 weeks

The total treatment time (Strandqvist 1944) as well as the total number of fractions (Ellis 1963) in fractionated *radiotherapy* are important Most studies on split-course radiotherapy have been *made on* human cancers or animal tumours Scanlon (1963) *however in* a casuistic report of split course radiotherapy for mediastinal *Hodgkin s disease reported* good regression of the lymphomas in the interval between the two *series after 1 350 R* Holsti (1966) found that the end results of split course *radiotherapy* of human cancers were not inferior to those after continuous *irradiation* though doses administered in the former cases were *larger Holsti* had often noted shrinkage of the tumours during the interval *between the two series* Sambrook (1963) claimed that treatment must be resumed *before recovery* of the mitotic activity in the tumour but added that this interval *is unknown* although he had never seen clinical evidence of renewed growth *in human* epitheliomas within the first 6 to 8 weeks after 2 000 to 2 500 R *given over* 2 to 3 weeks Du Sault (1954) in a study using different fractionation types of irradiation of spontaneous mammary carcinoma in mice reported that continuous treatment produces better results than split

Table 2

Comparison of patients without and patients with clinically diagnosed recurrences in relation to sex, age, clinical stage, histologic type and fractionation. — Median values and ranges are given for age and fractionation data

	Group A = Mediastinal recurrence not diagnosed clinically	Group B = Mediastinal recurrence diagnosed clinically
Males/females	6/5	5/3
Age (years) at first mediastinal treatment	31 (15—48)	27 (16—45)
Clinical stage at first mediastinal treatment: I	1	0
II	8	7
III	2	1
Histologic type in biopsy specimen		
Lymphocytic predominance	*3*	*1*
Nodular sclerosis	4	5
Mixed cellularity	4	2
Lymphocytic depletion	0	0
Treatment period (days)		
First series	22 (11—32)	25 (17—3[illegible])
Second series	13 (6—17)	12 (8—54)
Totally	71 (56—89)	76 (61—131)
Number of fractions		
First series	20 (12—24)	21 (16—24)
Second series	12 (6—16)	8 (8—16)
Totally	32 (19—36)	32 (24—33)
Interval (days) between the series	38 (31—49)	41 (31—47)

course therapy. The later the interval in split course treatment the better were the results. Though no direct correlation could be shown, the end results tended not to be as good *when the interval was long as when it was short*. SCANLON (1960) stated that the recovery time for tumours varies from patient to patient. FRIEDMAN et coll. (1967) produced an isoffect recovery curve indicating that Hodgkin's disease was capable of recovering from the effects of radiation. This recovery resembled that of carcinoma more closely than that of a radiosensitive lymphoma, such as *mycosis fungoides*.

The aim of the present retrospective investigation was to find out whether any difference in the target dose level was demonstrable between patients without and patients with *clinically diagnosed recurrences after initial radiation treatment* for mediastinal Hodgkin's disease. It was decided to study the lymph node group in the mediastinum (1) because this region is often involved early in

progressive disease (LANDBERG & LARSSON 1968 1969), (2) because the geometrics for mediastinal irradiation is fairly well defined and (3) because the results of therapy may be verified by roentgen examination

Material and Follow up A total of 246 patients with Hodgkin's disease were referred to the department during the period 1944—1960, and 149 of these who were previously untreated, were admitted Re examination of the biopsy specimens obtained before the beginning of treatment had invariably verified the diagnosis The patients had been followed up by the system described by LINDGREN (1962) The period covered by the present investigation allowed a follow up of at least 5 years from the commencement of treatment

The material was divided according to the clinical staging of the disease (JELLIFFE & THOMSON 1955 JELLIFFE 1965)

Stage I Lymph node involvement in only one main group but not including intra abdominal disease

Stage II Lymph node involvement of 2 or more groups in the upper or lower half of the body but not including intra abdominal disease

Stage III Generalized lymph node involvement — intra abdominal involvement — involvement of structures other than lymphatics — constitutional symptoms for which no other reasonable cause can be found

The material was divided according to the histologic type of lesion (LUKES et coll 1966) as follows lymphocytic predominance — nodular sclerosis — mixed cellularity — lymphocytic depletion Further details of the materials are given elsewhere (LANDBERG & LARSSON 1969 LANDBERG 1969)

Fifty six patients in all received radiation for mediastinal lymphomas (re irradiation for recurrence excluded) Lymphadenopathia at the site of treatment after the initial course of radiotherapy was regarded as a recurrence (KAPLAN 1966) Of the 56 patients 29 were excluded from this series one in whom the treatment had been given in one series only eighteen mostly with advanced disease who had been followed up for at the most one year after the treatment of the mediastinum and ten whose roentgenograms were not available for review In none of the remaining 27 patients had any mediastinal recurrence been diagnosed clinically within less than 26 months of the first irradiation of the mediastinum It was therefore decided to exclude a further eight patients who had been followed up for less than 26 months after the first mediastinal irradiation

The series thus consisted of 19 patients with mediastinal Hodgkin's disease followed up for 3 years or more after the first treatment of the mediastinum The examinations had revealed mediastinal recurrence in eight (group B) but not in the remaining eleven (group A) patients

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Comparison of patients without and patients with clinically diagnosed recurrences in relation to sex, age, clinical stage, histologic type and fractionation — Median values and ranges are given for age and fractionation data

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Totally		32 (19—36)	32 (24—33)
Interval (days) between the series		38 (31—49)	41 (31—47)

course therapy. The later the interval in split course treatment the better were the results. Though no direct correlation could be shown, the end results tended not to be as good when the interval was long as when it was short. SCANLON (1960) stated that the recovery time for tumours varies from patient to patient. FRIEDMAN et coll. (1967) produced an isoeffect recovery curve indicating that Hodgkin's disease was capable of recovering from the effects of radiation. This recovery resembled that of carcinoma more closely than that of a radiosensitive lymphoma, such as *mycosis fungoides*.

The aim of the present retrospective investigation was to find out whether any difference in the target dose level was demonstrable between patients without and patients with clinically diagnosed recurrences after initial radiation treatment for mediastinal Hodgkin's disease. It was decided to study the lymph node group in the mediastinum (1) because this region is often involved early in

progressive disease (LANDBERG & LARSSON 1968, 1969), (2) because the geometrics for mediastinal irradiation is fairly well defined and (3) because the results of therapy may be verified by roentgen examination

Material and Follow up A total of 246 patients with Hodgkin's disease were referred to the department during the period 1944—1960 and 149 of these who were previously untreated were admitted Re examination of the biopsy specimens obtained before the beginning of treatment had invariably verified the diagnosis The patients had been followed up by the system described by LINDGREN (1962) The period covered by the present investigation allowed a follow up of at least 5 years from the commencement of treatment

The material was divided according to the clinical staging of the disease (JELLIFFE & THOMSON 1955 JELLIFFE 1965)

Stage I Lymph node involvement in only one main group but not including intra abdominal disease

Stage II Lymph node involvement of 2 or more groups in the upper or lower half of the body but not including intra abdominal disease

Stage III Generalized lymph node involvement — intra abdominal involvement — involvement of structures other than lymphatics — constitutional symptoms for which no other reasonable cause can be found

The material was divided according to the histologic type of lesion (LUKES et coll 1966) as follows lymphocytic predominance — nodular sclerosis — mixed cellularity — lymphocytic depletion Further details of the materials are given elsewhere (LANDBERG & LARSSON 1969 LANDBERG 1969)

Fifty six patients in all received radiation for mediastinal lymphomas (re irradiation for recurrence excluded) Lymphadenopathia at the site of treatment after the initial course of radiotherapy was regarded as a recurrence (KAPLAN 1966) Of the 56 patients 29 were excluded from this series one in whom the treatment had been given in one series only eighteen mostly with advanced disease who had been followed up for at the most one year after the treatment of the mediastinum and ten whose roentgenograms were not available for review In none of the remaining 27 patients had any mediastinal recurrence been diagnosed clinically within less than 26 months of the first irradiation of the mediastinum It was therefore decided to exclude a further eight patients who had been followed up for less than 26 months after the first mediastinal irradiation

The series thus consisted of 19 patients with mediastinal Hodgkin's disease followed up for 3 years or more after the first treatment of the mediastinum The examinations had revealed mediastinal recurrence in eight (group B) but not in the remaining eleven (group A) patients

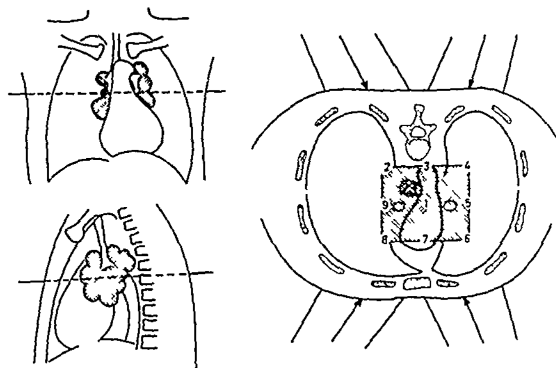


Fig. 1. Example of reconstructed contour and of irradiation geometry in mediastinal Hodgkin's disease.

Radiation data and estimation of the doses given. The treatment in all the nineteen patients had been given as split course therapy in two series (Table 2). Irradiation had been given in fifteen of the patients with 170 kV roentgen rays (HVL 0.9 mm Cu) and two ventral and two dorsal oblique portals, in one (No. 158) with 170 kV roentgen and two ventral portals, in one (No. 114) with 200 kV roentgen and rotation and in two (Nos. 94 and 128) with ^{60}Co and one ventral and two dorsal portals. One field was irradiated daily and the treatment was given 6 days a week.

Information about doses in clinical materials should be founded on individual dose planning made at the time of treatment. Such precise information is, however, seldom included in clinical reports, and the various ways employed in reporting doses given to patients make it difficult to compare the therapeutic results obtained in different clinics (ELLIS 1963).

In the present material chest roentgenograms taken at the beginning of treatment and later during follow up were available. Reconstructions were drawn for all the nineteen patients of a contour through the centre of the mediastinal lymphoma by means of the roentgenograms that had been obtained at the beginning of treatment (Fig. 1). The roentgenographic enlargement factor was taken as 1.10 for a.p. views and 1.15 for lateral views. The error in such reconstructions

may be considerable in the lateral parts of the contour but can be kept within reasonable limits in the medial parts In ten patients the reconstructed contours were checked either with contours made at the time of treatment or with those obtained at a review The reconstructed contours in the medial parts in these ten patients agreed well the difference in a p distance was found to be within ± 1 cm It is apparent from Fig 1 that only the medial parts of the contours are of interest in the further discussion

The patient records contained information on the surface absorbed dose type and energy of radiation geometric field sizes and angle of incidence The dose delivered to nine different points (Fig 1) was calculated with the assumption that the volume treated was comparable to water-equivalent tissue from the point of view of radiation absorption (Clinical Dosimetry ICRU Report 10d, 1963) The dose to the target volume varied noticeably in the 4 portal technique (Fig 1) In addition the lateral parts of the target volume were prone to fall outside the radiation fields even with slight variation of the angle of incidence and thus a minor variation in the set up could result in considerable changes in the distribution of the dose delivered The patients were however young mostly in good general condition and presumably cooperated well Roentgenologically the regression of mediastinal lymphomas is centripetal and this was noted in sixteen of the nineteen patients by the time the second series was started When it occurs, the degree of reproducibility of the geometry probably becomes less critical

The treatments have thus been expressed as the doses to the centres of the target volumes and disregard the dose variation in the peripheral parts of the target volumes

Dose measurements were made in phantoms in order to estimate the error involved by the assumption that the irradiated tissue was water equivalent A volume in a Machlett Alderson Rando Phantom (Machlett Laboratories Inc Springdale Connecticut U S A) with skeleton and simulated lung tissue was irradiated with the 4 portal technique (Fig 1) A phantom of the same dimensions but consisting only of water equivalent material was then made and a corresponding volume was irradiated Dose measurements were made with individually calibrated thermoluminescence dosimeters (lithium borate in teflon) The measured dose delivered to the centre of the target volume in the Alderson phantom was 111 ± 2 rad S D (overall uncertainty) based on the maximum absorbed surface dose of 100 rad for a single field The corresponding figure for the homogeneous water equivalent phantom was 106 ± 2 rad S D The calculated dose to the centre of the target volume was 100 rad If it be accepted that this experiment can be used as a guide in the evaluation of the doses calculated for the patients, then a correction of the calculated dose to the centre

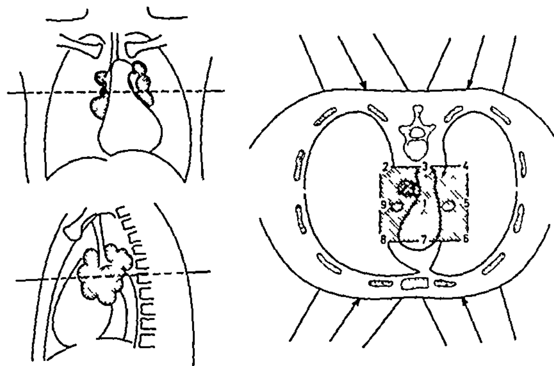


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Table 3

Treatment data for each one of the patients and interval between the first mediastinal irradiation and clinical detection of recurrence in patients with and without mediastinal recurrences — L denotes living patients and D those who died

	Patient No	Dose to centre of target volume (*rad*)	Total treatment period (days)	Total number of fractions	Follow up after first mediastinal irradiation (*months*)	Mediastinal recurrence after first irradiation (*months*)
Group A Mediastinal recurrence not diagnosed clinically	29	4 100	89	37	159 L	
	47	3 600	82	36	122 D	
	58	4 400	89	36	175 L	
	72	3 900	83	36	150 L	
	74	5 500	68	30	43 D	
	85	3 700	71	37	137 L	
	94	5 000	67	30	54 L	
	112	4 200	61	28	104 L	
	114	3 600	60	19	50 L	
	128	4 400	74	33	60 L	
	158	3 400	56	30	60 L	
Group B = Mediastinal recurrence diagnosed clinically	6	3 500	83	33	197 D	92
	11	3 900	131	32	39 D	26
	38	3 400	77	32	83 D	51
	40	3 400	75	30	56 D	51
	50	3 100	80	32	81 D	75
	95	3 000	61	28	95 D	73
	139	3 500	75	32	93 L	73
	150	4 300	66	24	58 D	35

of the mediastinal irradiation The mediastinal recurrence was the first new sign of Hodgkin's disease after the irradiation of the mediastinum in two of the patients in group B (Nos 6 and 139)

Comparison between patients without and patients with recurrence in relation to dosage A review of the roentgenograms obtained before completion of the irradiation of the mediastinum revealed changes in the pulmonary parenchyma or pleurae in five of the nineteen patients The changes were located outside the irradiation fields in three patients (Nos 74 95 and 150) Such changes though slight were seen within the irradiation field in two patients (Nos 112 and 114)

The data for each one of the patients are assembled in Table 3 giving the calculated dose to the centre of the target volume the treatment period the

of the target volume by reason of heterogeneous tissue should be in the order of + 10 %. We have, however, only indicated the calculated dose in the text. The correction factor for determination of the absolute dose should then be 1.1. A similar figure has been given by JACOBSON & KNAUER (1956), who made measurements with ionization chambers in a phantom man made of presdwood and having a cork lung.

Results and Discussion

Comparison between findings in patients without recurrence and patients with recurrences irrespective of dosage. The second treatment series was prolonged to 54 days in one patient (No. 11-B), but otherwise the patients in group A and in group B were comparable (Table 2) as regards distribution according to sex, age, clinical stage, histologic type and fractionation. The height of the irradiation fields was 10 to 18 cm in the patients in group A and 11 to 22 cm in the patients in group B.

Three of the patients in group A (Nos 47, 74 and 114) and one of the patients in group B (No. 11) had also extra mediastinal manifestations which had not been treated when the mediastinal irradiation was completed.

Roentgen examination before the beginning of the second series revealed no abnormality in four of the patients in group A (Nos 29, 72, 74 and 94) and in one of the patients in group B (No. 150).

The mediastinal recurrences were diagnosed 26 to 92 months (mean 60) after the irradiation of the mediastinum. Of the eleven patients in group A, eight had been followed up for 5 years or more, and six for more than 8 years after the beginning of the mediastinal irradiation. In the series of KAPLAN (1966) and of SEYDEL et coll. (1967) recurrences and extensions tended to occur within 2 to 3 years of the beginning of treatment of Hodgkin's disease. In the present series, at first all patients with a follow up of at the most a year were excluded; among the remainder recurrences were not diagnosed until 26 months or more after irradiation of the mediastinum had been started.

Treatment with cytotoxic drugs had been initiated before the first irradiation of the mediastinum in three of the patients in group A (Nos 85, 114 and 158) but in none of the patients in group B. Later, five more patients in group A and all patients in group B received cytotoxics. Such treatment had been started in five of the eight patients in group B before the mediastinal recurrence had been diagnosed.

Further manifestations of Hodgkin's disease at other sites were diagnosed later in six of the patients in group A at 4 to 68 months (mean 32) and in all the eight patients in group B at 1 to 191 months (mean 42) after the beginning

ment period (Fig 2) and to the number of fractions (Fig 3) It is apparent that for a given number of days or with a given number of fractions recurrences were more common among patients who had received smaller doses For a given dose level the frequency of recurrences seemed to diminish with a decreasing number of days and a decreasing number of fractions It was not possible however, to analyze the material in order to obtain a fractionation curve

The presence or absence of a recurrence in the present material thus tended to vary with the calculated dose to the centre of the target volume in relation to the treatment period and to the number of fractions but not with any other of the factors studied The treatments were administered within (median) 75 days and in (median) 32 fractions and it seemed that in these intervals the dose to the centre of the target volume should not be below 3 600 rad if it is to be effective The dose would be about 4 000 rad with the correction factor of 1 1 for inhomogeneous tissue

Autopsy was performed in one of the group A patients who had died (No 47) the cause of death was Hodgkin s disease The examination revealed signs of the disease in a node in the mediastinum

Acknowledgements

The authors wish to thank I Bang of the Department of Roentgen Diagnosis for help with the re evaluation of the roentgenograms The work was supported by grants from the Swedish Cancer Society

SUMMARY

Doses delivered to the centres of the target volumes were calculated retrospectively in nineteen patients followed up for at least 3 years after split course irradiation for mediastinal Hodgkin s disease There were eleven patients without recurrences who were compared with eight patients in whom recurrences had been diagnosed clinically The development or absence of a recurrence seems to depend mainly on the size of the dose to the centre of the target volume

ZUSAMMENFASSUNG

Die um Zentrum des Bestrahlungsgebietes gelieferten Dosen in neunzehn Patienten mit Hodgkinscher Krankheit des Mediastinums die fur ein Minimum von 3 Jahren nach fraktionierter Tiefenbestrahlung unter Kontrolle gehalten werden konnten wurden retrospektiv berechnet Ein Vergleich konnte zwischen elf Patienten die rezidivfrei waren, und acht Patienten, die klinisch Rezidive hatten angestellt werden Das Auftreten von Rezidiven scheint im wesentlichen von der Grosse der Dosis im Zentrum des Bestrahlungsgebietes abhangig zu sein

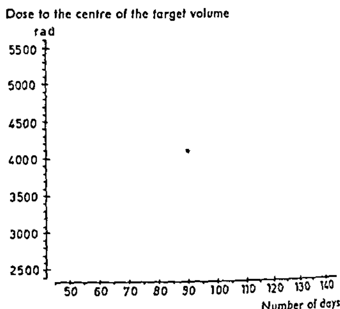


Fig 2 Dose to the centre of the target volume distributed at different treatment periods (days) for patients without recurrence (●) and for those with clinically diagnosed mediastinal recurrences (×)

number of fractions and the follow up and, for the patients in group B, also the recurrence free interval

The dose to the centre of the target volume for patients without and with clinically diagnosed recurrences is recorded in Figs 2 and 3, related to the treat

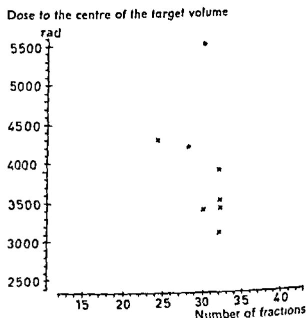


Fig 3 Dose to the centre of the target volume distributed with different number of fractions for patients without recurrence (●) and for those with clinically diagnosed mediastinal recurrences (×)

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RÉSUMÉ

Les auteurs ont calculé rétrospectivement les doses délivrées au centre des volumes cibles chez 19 malades suivis pendant trois ans au moins après irradiation fractionnée pour maladie de Hodgkin médiastinale. Ils ont comparé onze malades sans récidive et huit malades chez lesquels on avait fait le diagnostic clinique de récidive. L'apparition ou l'absence d'une récidive paraît dépendre surtout de la dose calculée au centre du volume cible.

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FROM THE DEPARTMENT OF RADIATION PHYSICS (DIRECTOR PROF K LIDEN), UNIVERSITY OF LUND, SWEDEN

DOSIMETRIC STUDIES OF MANTLE FIELDS IN COBALT 60 THERAPY OF MALIGNANT LYMPHOMAS

by

GUDRUN SVAHN-TAPPER

During the past few years an increasing number of radiotherapists have advocated a particular type of radiation treatment for certain forms of lymph node diseases, a significantly larger portion of the body being subjected to radiation than was previously customary. These conditions which were formerly regarded as generalized are now considered to be initially local and therefore possibly curable (CRAVER 1954, KAPLAN 1962, 1966, EASSON & RUSSEL 1963, PETERS 1966 and MUSSHOFF & BOUTIS 1968). It has also been demonstrated that the spread is often at first to the adjacent lymph node groups (JELLIFFE 1965, NEWALL 1965, KAPLAN 1966, ROSENBERG & KAPLAN 1966, LANDBERG & LARSSON 1968, LANDBERG 1969). The best results of treatment should therefore be attained if the clinically involved lymph node groups as well as adjacent groups are irradiated. The submandibular, cervical, supraclavicular, infraclavicular, axillary and mediastinal lymph node groups are consequently all irradiated in supradiaphragmatic conditions. The simplest technique for such treatment is the use of two large opposing anterior and posterior fields with lead blocks interposed to shield tissues not to be irradiated. Large source to skin distances are necessary and the treatment fields are irregular in shape.

Submitted for publication 26 June 1969

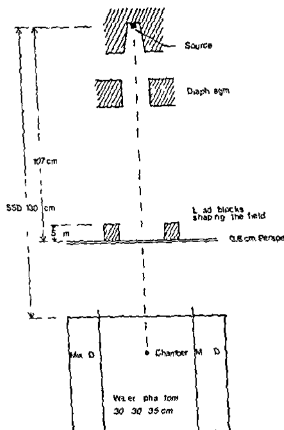


Fig 1 Schematic drawing of apparatus for the determination of depth doses to different parts of the mantle field The clinical situation is simula ed by exchanging the water phantom and mix D blocks for the thorax phantom.

This report describes isodose charts for a sagittal plane in the centre of the fi ld with and without a beam flattening filter and for transverse planes at the levels of the jugulum and the pulmonary hila A comparison between planned dose distribution and measured dose at certain points in a thorax phantom i also presented

Material and Methods

Experimental equipment The measurements were carried out with a Siemens Gammatron III which has a cobalt 60 source with a diameter of 1 5 cm The cobalt 60 unit is equipped with a block diaphragm its anterior part being 23 8 cm from the source The edge of the light beam was adjusted to match the 50 % isodose line at 0 5 cm depth for a field size of 10 cm × 10 cm All

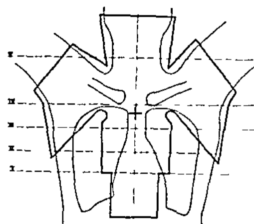
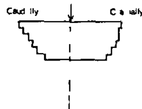


Fig 2 Mantle field with reference to thorax phantom Dotted lines indicate planes where dose planning and measurements are carried out



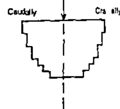


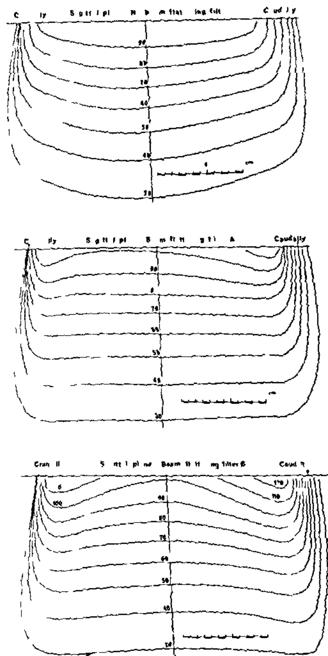
Fig 3 The two beam flattening filters A and B are made of perspex with a density of 1 18 kg/cm³ source filter distance 30 2 cm

measurements were carried out at an SSD of 130 cm The beam direction was vertical

Phantoms used in the measurements All depth dose measurements were performed in a 30 cm × 30 cm × 35 cm water phantom, and with mix D as side scattering material where the phantom was not sufficiently large The film exposure for dose determination was made in a polystyrene phantom 35 cm × 35 cm × 30 cm, also with mix D as side scattering material The thorax phantom consisted of mix D and contained a skeleton, the arms being above the head with the neck extended The lungs were filled with sawdust to a density of 0 25 g/cm³, which according to DAHL & VIKTERLOF (1960) is the radiation equivalent of an air filled lung Unfortunately, the chest wall of the phantom was too thick and the apices of the lungs terminated at the jugulum instead of at Th2

Beam flattening filters The beam flattening filters used in the cranial caudal direction were made up of perspex plates, 0 5 cm thick, with a density of 1 18 g/cm³ The source filter distance was 30 2 cm (see Fig 3)

Shape and size of the mantle field The mantle field was formed of 5 cm lead blocks placed on a 0 8 cm perspex plate 107 cm from the cobalt source (Fig 1) The sides of the lead blocks were vertical and the shape and size of the mantle field were adjusted to simulate a mantle treatment, the size of the field before the lead blocks were interposed was 38 cm × 40 cm The mantle



F g 4 Isod se l arts for the *sagittal plane* i i th middle of the mantle f eld without beam flatten ng filte (upper) and re pe ti ely with beam flattening filter A (middle) and filter B (low rn t)

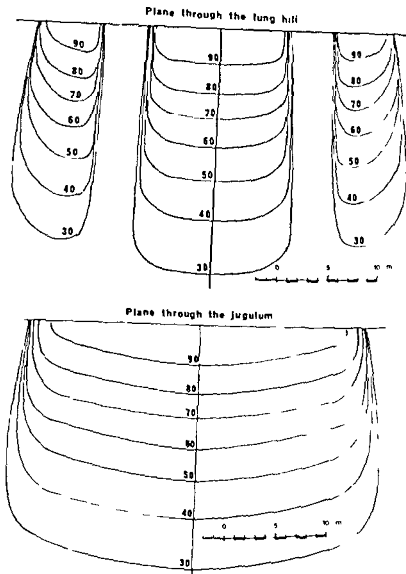


Fig 5 Isodose charts for transverse planes according to fig 2 no beam flattening filter in either direction

field was so formed (Fig 2) that the light beam indicated a border of 1 cm outside the target area, projected vertically onto the surface of the phantom where the target area included all supradiaphragmal lymph nodes from the mastoid process to Th12 The primary field extension outside the target was 3 cm, 2 cm of which were shielded by lead on the cranial and lateral borders The broad penumbra was left undiminished on the caudal border to facilitate the addition of abdominal fields With depth dose measurements for square fields, the size of the field was defined by the diaphragm of the cobalt unit

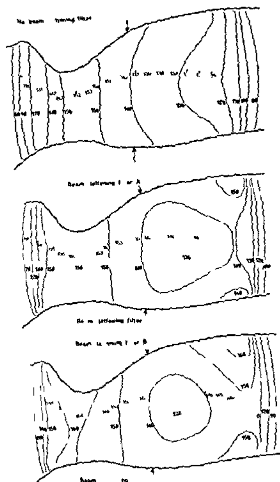


Fig 6 Dose planning for anterior and posterior fields in the sag ttal plane through the middle of the phantom re pectively without and with beam flatten ng filters ↘ indicates dose value meas red with con l n c chambers Th trachea is filled ith paraffin pellets

Measuring procedure Depth dose measurements for all the fields were made with the aid of a cable connected ionization chamber the air volume of which was 1 0 cm^3 An ionization chamber of air volume 0 1 cm^3 cable connected was al o used in the determination of depth doses for field sizes 10 cm × 10 cm and 17 cm × 17 cm All the ionization chambers were calibrated free in air again t a substandard chamber which had in turn been calibrated at the Standard Laboratory of the Swedi h Radiation Protection Institute When the ionization

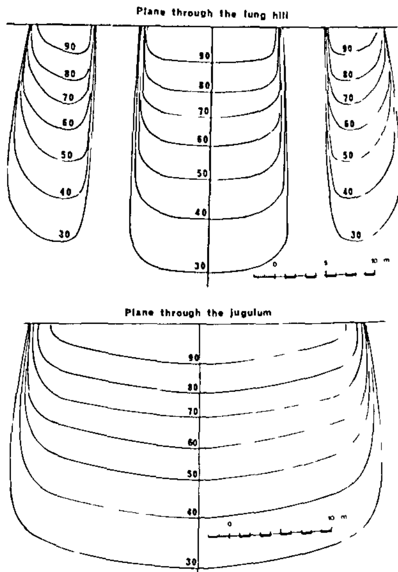


Fig. 3. Isodose charts for transverse plane according to fig. 2, no beam flattening filter in either direction

field was so formed (Fig. 2) that the light beam indicated a border of 1 cm outside the target area projected vertically onto the surface of the phantom where the target area included all supradiaphragmal lymph nodes from the mastoid process to Th12. The primary field extension outside the target was 3 cm, 2 cm of which were shielded by lead on the cranial and lateral borders. The broad penumbra was left undiminished on the caudal border to facilitate the addition of abdominal fields. With depth dose measurements for square fields, the size of the field was defined by the diaphragm of the cobalt unit.

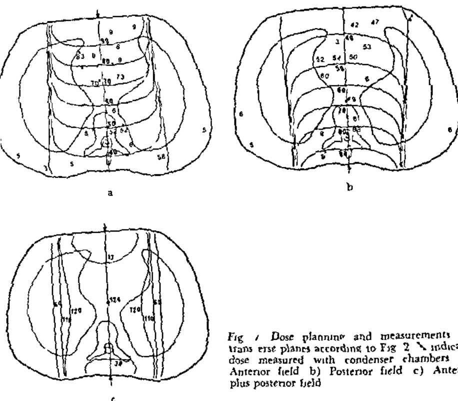


Fig 4 Dose planning and measurements for transverse planes according to Fig 2 ↘ *indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field*

Results and Discussion

Calculation of depth dose The depth dose for SSD = 130 cm has been calculated for field sizes 0 cm 10 cm × 10 cm, 17 cm × 17 cm and 20 cm × 20 cm according to the British Journal of Radiology (1961), Suppl No 10 The tissue/air ratio measured by GUPTA & CUNNINGHAM (1966) was used in the calculation Depth dose calculations for the mantle field were made according to the method of CLARKSON (1941) The field was then divided into sectors of 5 The scatter function according to GUPTA & CUNNINGHAM (1966), is given as

$$S(r_x\, d\, F) = 100\left(\frac{F+x}{F+d}\right)^2 \left[T(r_d\, d) - \exp\{-\mu(d-x)\}\right]$$

where the scatter function is defined as the dose due to scattered radiation at points along the central axis of the beam per 100 rad from primary radiation

chambers are used for dose measurements at different depths in a phantom the photon spectrum differs considerably from the spectrum free in air, and the secondary radiation is almost isotropically incident on the ionization chamber Photon spectra for different depths and for different field sizes are reproduced in the diagram of BRUCE & JOHNS (1960)

According to calculations, the equivalent area to the mantle field is approximately 25 cm × 25 cm The calibration constant for the ionization chamber of volume 1 0 cm³ determined free in air varied less than 2 % for HVL 0 2 mm Cu to HVL 14 6 mm Cu Thus, no corrections for energy dependence were made and the possible direction dependence for cobalt radiation could be ignored In measurements with the ionization chamber of volume 0 1 cm these two factors were eliminated through a comparative measurement using the same field size at a SSD of 100 cm for which depth doses are known (Brit J Radiol 1961) The difference in the depth dose measurements of the small and large ionization chambers was 2 % of the measured value for a depth of 20 cm, after standardizing to 5 cm depth Depth dose measurements in water for mantle fields were also carried out by means of a Fricke dosimeter The volume of the dosimeter was 4 ml

Depth dose measurements made in eight different radiation directions, distributed evenly over the mantle field, form the basis for the determination of isodose charts Measurements were carried out with the aid of the cable connected ionization chamber of volume 1 0 cm³ Supplementary measurement were made with condenser chambers of volume 0 3 cm³ at depths of 5 cm and 15 cm Film dosimetry was used for further help in drawing the isodose lines It proved to be impossible to obtain a linear relationship between film density and exposure within the whole mantle field, since the energy spectrum differs between the central and distal parts of the field Isodensity lines were therefore used only for the distal parts of the field, where an explicit relationship between density and exposure could be obtained In the other parts of the mantle field they were used only as an aid in drawing the isodose lines between the dose values measured by the ionization chamber

The thorax phantom was dose planned centrally in a sagittal plane both with and without beam flattening filter as shown in Fig 6, and in five transverse planes as seen in Figs 7—11 Correction for irregular body contour was made with the isodose shift method (DUTREIX & DUTREIX 1962) In the axillary and submandibular regions the isodoses were shifted in the direction of the divergence of the local beam The dose measurements in these transverse planes as well as in the oesophagus were carried out with the aid of condenser chambers

All figures in the isodose charts and dose planes are written on the higher dose side of the isodose line

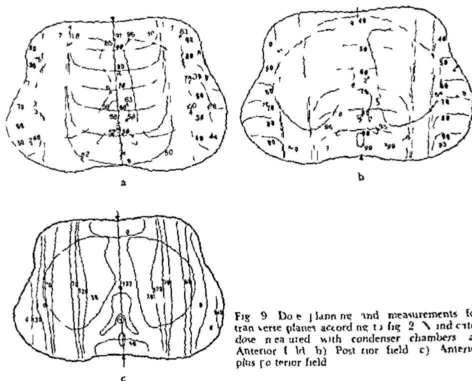


Fig 9 Dose planning and measurements for transverse planes according to fig 2 X indicates dose measured with condenser chambers a) Anterior field b) Posterior field c) Anterior plus posterior field

For the point below the centre the calculated dose at a depth of 10 *cm* is 62.5 % and the dose measured with the ionization chamber 61.8 % If the field is 4 cm longer in a caudal direction the calculated dose at 10 cm is 63.1 % and if the field is shortened 4 cm caudally the calculated dose becomes 61.3 %

Comparison between calculated and measured depth dose A comparison is made in Table 1 between measured and calculated values of depth doses, the former being standardized to those calculated at 5 cm The mean values of measurements with two ionization chambers are entered in the table for the fields 10 cm × 10 cm and 17 cm × 17 cm The uncertainty in the Fricke measurements at a depth of 14.6 *cm* is ± 5 % of the depth dose value The difference in measured and calculated values for greater depths may possibly depend on the difference in the back scattering material At 20 cm depth 10.7 cm of phantom material lay behind the ionization chamber while the tissue/air ratios and scatter functions were determined in a water phantom that was 60

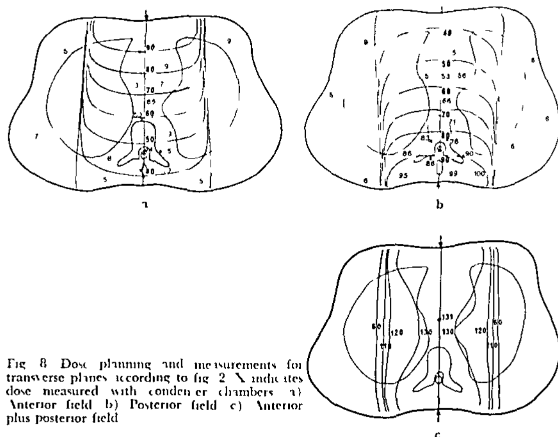


Fig. 8. Dose planning and measurements for transverse planes according to fig. 2. X indicates dose measured with condenser chambers. a) Anterior field. b) Posterior field. c) Anterior plus posterior field.

alone at the position of the maximum dose in the phantom, x is the depth of maximum dose, r_x the radius a circular beam would have at depth x, F is the SSD, $T(r_x, d)$ is the tissue/air ratio for depth d in the phantom and μ is the narrow beam attenuation coefficient for the phantom material.

Since the tissue/air ratio is independent of SSD, $S(r_x, d, 130)$ may be written

$$S(r_x, d, 130) = \frac{\left(\frac{130.5}{130+d}\right)^2}{\left(\frac{80.5}{80+d}\right)^2} \cdot S(r_x, d, 80)$$

$$\text{where } r_x = \frac{80.5}{80+d} \cdot \frac{130+d}{130.5} \cdot r_x$$

Scatter functions for SSD = 80 cm have been tabulated by the same authors. Calculation of the depth dose is made for the centre of the field (Table 1) and for a point 11.6 cm below the centre. The scatter function for the mantle field has the same value as for a square of 25 cm × 25 cm.

Table 1

Calculated and measured percentage depth doses for SSD = 130 cm — The measured depth doses are standardized to the calculated doses at 5 cm

Depth cm	Field sizes						Mantle field equivalent to square 25 cm × 25 cm		
	10 cm × 10 cm		15 cm × 17 cm		20 cm × 20 cm				
	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Measured Fricke dosimeter
0 5	100	93 6	100	98 9	100	98 5	100	99 2	
1 0								99 2	
2 0	94 9		95 5		95 7				
5 0	81 9	81 9	83 9	83 9	84 4	84 4	84 8	84 8	84 9
9 8							67 4		67 4
10 0	60 3	60 5	64 1	64 4	65 2	65 3	66 6	67 0	
14 6							51 7		52 5
15 0	43 4	44 4	47 9	49 1	49 2	49 4	51 1	50 6	
20 0	31	30 1	35 8	36 4	37 2	36 8	39 1	37 7	

Table 2

Comparison of the dose rates in the central line of mantle fields of different sizes

Depth cm	Field suiting the phantom including bula	Field suiting the phantom excluding bula	Field suiting a small patient including bula	Field suiting a tall patient including bula
	100	98 5	98 9	101
[illegible]	1 0	49 6	50 0	52 0

has a greater antero-posterior thickness in the submandibular and mediastinal region as compared to the jugulum level the filters are overcompensating to produce as homogeneous a dose as possible to the whole target volume The depth dose at less than 20 cm depth in the centre of the field with beam flattening filters does not differ from the depth dose without filter

Comparison of planned and measured dose in a thorax phantom The upper charts a and b in Figs 7 8 9 and 10 indicate the planned and the measured dose values respectively for both the anterior and the posterior treatment field in the transverse planes I to IV as in Fig 2 Measurements and dose

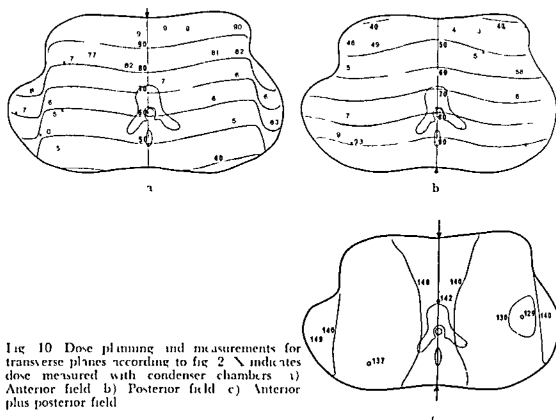


Fig. 10. Dose planning and measurements for transverse planes according to fig. 2. X indicates dose measured with condenser chambers. a) Anterior field. b) Posterior field. c) Anterior plus posterior field.

cm deep. The measured values have been used in the isodose charts but standardized to 100 % at 0.5 cm depth.

Dose rate variation according to the shape and size of the mantle fields. The mantle field must be modified individually in the treatment of patients and therefore the shape and size may differ from one patient to another. The measurements were made in a water phantom representing two extreme fields, the one in a child of 12 years (157 cm, 41 kg) and the other in a male adult (190 cm, 100 kg). The measurements were carried out in the centre, and the back scatter material was the same for all fields. A comparative measurement in which the hilar region was covered with lead blocks was also performed. The results are presented in Table 2.

Isodose charts. Isodose charts centrally in a sagittal plane are shown in Fig. 4, and for transverse planes at respectively the hilar and jugulum levels in Fig. 5. The depth dose in the transverse planes was adjusted to the depth dose in the sagittal plane at the corresponding level. Two beam flattening filters (cf. Fig. 3) were made for the sagittal plane. Since the patient always

Table 1

Calculated and measured percentage depth doses for SSD = 130 cm — The measured depth doses are standardized to the calculated doses at 5 cm

Depth in cm	Field sizes						Mantle field equivalent to square 25 cm × 25 cm		
	10 cm × 10 cm		17 cm × 17 cm		20 cm × 20 cm				
	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Calculated	Measured ionization chamber	Measured Fricke dosimeter
0.5	100	98.6	100	98.9	100	98.5	100	99.[illegible]	
1.0								99.2	
2.0	94.9		95.5		95.7				
5.0	81.9	81.9	83.9	83.9	84.4	84.4	84.8	84.8	84.8
[illegible]8							67.4		67.4
10.0	60.3	60.5	64.1	64.4	65.2	65.3	6[illegible].6	67.0	
14.[illegible]							51.7		5[illegible].5
15.0	43.4	44.4	47.9	49.1	49.2	49.4	51.1	50.6	
20.0	31.2	30.7	35.8	36.4	37.[illegible]	36.8	39.1	37.7	

Table 2

Comparison of the dose rates in the central line of mantle fields of different sizes

Depth cm	Field suiting the phantom including hila	Field suiting the phantom excluding hila	Field suiting a small patient including hila	Field suiting a tall patient including hila
	100	93.5	98.9	101.[illegible]
1[illegible]	51.0	49.6	50.0	5[illegible].0

has a greater antero posterior thickness in the submandibular and mediastinal regions as compared to the jugulum level the filters are overcompensating to produce as homogeneous a dose as possible to the whole target volume The depth dose at less than 20 cm depth in the centre of the field with beam flattening filters does not differ from the depth dose without filter

Comparison of planned and measured dose in a thorax phantom The upper charts (a and b) in Figs 7 8 9 and 10 indicate the planned and the measured dose values respectively for both the anterior and the posterior treatment fields in the transverse planes I to IV as in Fig 2 Measurements and dose

Fig. 11. Dose planning and measurements for two phantoms of the neck differing in size. X indicates the dose measured with condenser chambers. Air in trachea.

planning were carried out for two different phantoms of the neck, since the neck contour of patients often differs considerably (Fig. 11). The trachea was filled with paraffin pellets in the oesophagus measurements (Fig. 6). Measurements made with air in the trachea, where the diameter of the air cavity was 16 mm, produced a 7 % increase in the hypopharynx and the oesophagus versus the subcutaneous maximum dose in the centre of the ventral field. Thus, to obtain the best possible interpretation of the measured values in the hypopharynx and the oesophagus, the position of the catheter with ionizing chambers, in relation to the trachea, should always be determined with the aid of a film exposed during the treatment.

A statistical analysis of the accuracy of the measured values is difficult to carry out, since systematic errors are introduced. For example, no correction was made for inhomogeneous tissue and the correction for the body contour was made with the isodose shift method, even when the angle of incidence was more than 45°. No consideration was made for the smaller mass of the side scattering material in the thorax phantom measurements as compared with the water phantom measurements. The accuracy of the ionization chamber

Fig. 12. Dose planning in the hilar region for anterior and posterior fields with correction for lung density 0.25 g/cm³. (Cf. fig. 8 c.)

measurements is ± 4 % of the measured value this figure includes an energy dependence of about 1 %

Lung correction at the hilar level To calculate the lung correction the ionization chamber measurements were performed for the anterior field at four different lung depths at the hilar level If the depth dose curve in water for the mentioned level in the mantle fields is drawn as a straight line in a semi logarithmic diagram for depths greater than 5 cm the measurements in the lung plotted in the same diagram follow a line with a slope of 0 6 compared with the depth dose decrease in water

The isodose distribution at the hilar level with lung correction is indicated in Fig 12 (cf Fig 8c)

Conclusion

In spite of all uncertainties introduced in the treatment planning of the thorax phantom the agreement with the dose measured at certain points is surprisingly good In the mantle treatment of patients it should therefore be sufficient to *control the dose in the sagittal plane by measurements* in the hypopharynx and oesophagus for checking the effect of the field size the patient s size and the possible movements of viscera between the two treatment positions

To obtain as homogeneous a dose as possible in the whole target area a beam flattening filter should be used in a cranial caudal direction with an individual filter in the cervical region The *hilar* regions of the lungs should be shielded with lead during some of the treatments to prevent a too high dose to the lungs

SUMMARY

The dosimetry for mantle fields in the treatment of supradiaphragmatic malignant lymphomas with cobalt 60 was analysed Isodose charts for the mantle field were constructed in sagittal and in different transverse planes Studies of the agreement between planned and measured doses in a thorax phantom were performed both with and without beam flattening filters

ZUSAMMENFASSUNG

Die dosimetrischen Grundlagen fur Mantelfelder wie diese bei der Behandlung von supradiaphragmatischen malignen Lymphome mittels Cobalt 60 hier ublich sind wurden studiert Es wurden Isodosenplane fur *Mantelfelder* in der Sagittalebene und in verschiedenen Transversalebenen konstruiert An einem Thoraxphantom wurde das Über instimmen der geplanten Dosen und der wirklich gemessenen Dosis mit und ohne dem Gebrauch von Ausgleichfiltern gepruft

RÉSUMÉ

L'auteur a analysé la dosimétrie des champs en mantelet pour le traitement des lymphomes malins sus-diaphragmatiques par le cobalt 60. Elle a construit des courbes isodoses pour le champ en mantelet dans un plan sagittal et dans différents plans transversaux. Elle a étudié la concordance entre les doses prévues et les doses mesurées sur un fantome de thorax avec et sans filtre compensateur du faisceau.

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EFFECT OF SELECTIVE TUMOR HEATING ON THE LOCALIZATION OF ^{131}I FIBRINOGEN IN THE WALKER CARCINOMA 256

I. Heating by immersion in warm water

by

EDMUND S. COPELAND

Intravenously administered ^{131}I labeled fibrinogen or antibody to fibrin localizes preferentially enough in some transplantable rat tumors that it can be used for effective tumor radiation therapy (DAY et coll. 1959, BALE et coll. 1960). Localization also occurs quite commonly in human tumors, sometimes to an extent that the use of this method for therapy seems feasible. However, attempts at therapy have *not produced* prolonged remissions (MCCARDLE et coll. 1966). In many human tumors and in some transplantable rat tumors, in particular the Walker *carcinoma 256*, *the localization* of radioiodinated fibrinogen is not remarkable.

The purpose of the present work is to investigate methods by which the localization of ^{131}I fibrinogen in the Walker carcinoma 256 may be enhanced, with the hope that successful *techniques* might serve as useful adjuncts to human cancer therapy with radioiodinated *fibrinogen*. *It has* been suggested that deposition of fibrin results from the invasive growth of the tumor and is due perhaps to

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an inflammatory reaction of the host tissues or to simple injury of blood vessels of supporting stroma or invaded tissues (BALE et coll. 1960).

In this laboratory, the localization of ^{131}I fibrinogen in two transplantable rat tumors has been studied extensively. In the course of this investigation, localization will refer to the amount of ^{131}I activity found in a specific site and presumably represents the result of a steady deposition and destruction of fibrin. SHAFFER (1962) has shown that about 20 % of the injected radioactivity localizes in a 2.5 gram Murphy-Sturm lymphosarcoma 18 hours after intravenous administration of ^{131}I fibrinogen. A 3 gram Walker tumor, on the other hand, as reported later, localizes only 1 % of the injected dose 3 days after ^{131}I fibrinogen injection. If the percentages of the remaining whole body activity found in the tumors at these times are calculated for these two tumors, one obtains 30 % and 8 % for the Murphy-Sturm and Walker tumors, respectively. According to HIRAMOTO et coll. (1960), the small amount of localization in the Walker tumor might be explained in part on the basis of its slower growth rate as compared to the Murphy tumor. However, studies by SOARDI (1959) suggest that rats bearing the Walker tumor have increased plasma fibrinolytic activity and slightly reduced blood coagulability. VARON & SPAR (1965) have demonstrated that enhancement of the coagulation mechanism of the host increases fibrinogen localization in the Murphy-Sturm lymphosarcoma. BALE et coll. (1962) have shown that when rats bearing the Walker tumor are given epsilon aminocaproic acid (EACA) in their drinking water, a marked increase in tumor fibrin localization occurs. MUTSCHLER (1963, 1964) has extensively investigated the effect of EACA as a fibrinolysis inhibitor. Her results were corroborated in this investigation and are discussed in detail below.

A simple model can serve as the basis for experimentation: tumor fibrin localization equals depositions minus removal. Its removal from the tumor site involves fibrinolytic processes, thus systemic inhibition of fibrinolysis should result in increased tumor fibrin localization. Tumor fibrin deposition may be hypothetically divided into two phases: fibrinogen extravasation due to a localized inflammatory reaction or to invasive damage to vasculature, and fibrinogen transformation to fibrin by the coagulation mechanism. It seems reasonable then that at least three means could be employed to increase the amount of fibrin localized in the Walker and other tumors: (1) inhibition of the fibrinolytic system of the host, (2) enhancement of any inflammatory reaction occurring at the tumor site, and (3) selective damage to tumor tissue.

If tumor cells and cells of the tumor stroma, such as mast cells and capillary endothelial cells, could be selectively damaged in some way, mediators of the inflammatory reaction such as histamine might be released at the site of the insult and increased fibrinogen extravasation would occur. If tissue thromoplastin

were also released from the damaged cells thus extravasated fibrinogen should clot at the site of injury It is also possible that selective tumor damage might directly injure the vasculature of the supporting stroma and in this way increase fibrinogen extravasation

In 1935 WARREN studied the effects of artificially induced fever upon hopeless tumor cases Infrared heat lamps were used to induce fever (rectal temperature 41 5 C) for periods up to 21 hours After such treatment tumor growth was temporarily retarded and the patient s life was prolonged His work shows that tumor tissue is more susceptible to destruction by heat than are the tissues in which it grows CRILE JR (1963) has reported that heating certain melanomas implanted in the feet of mice to 44° C for from 30 to 40 minutes destroyed a high proportion of the tumors without damage to the feet

Materials and Methods

Rat fibrinogen Fraction 1 was obtained from fresh oxalated rat plasma by alcohol addition in the cold (ice salt bath) according to method 6 of COHN et coll (1946) modified by the omission of acetate buffer The procedure is essentially that reported by SHAEFFER (1964)

Labeling of fibrinogen with ¹³¹I The protein iodination method of HELMKAMP et coll (1960) using iodine monochloride as modified by BALE et coll (1966) to include the use of catalase to eliminate H_2O_2 was followed throughout these studies

¹³¹I labeled rat albumin Rat albumin was isolated from fresh rat serum according to a modified version of the acidified methanol technique of MICHAEL 1962) This twice recrystallized albumin was then radioiodinated using the technique described for fibrinogen iodination The purity of the ^{131}I albumin preparation was then tested by several methods Only 1 5 % of the radioactivity of an aliquot was bound to proteins precipitable with thrombin More than 97 % of the radioactivity of an aliquot precipitated in 20 % trichloracetic acid Nearly 93 % of the radioactivity of ^{131}I albumin spiked plasma or serum samples remained in solution when one third volume saturated ammonium sulfate was added Electrophoresis studies indicated that not more than 5 % of the sample radioactivity could be fibrinogen bound

Experimental animals Sprague Dawley female rats (Holtzmann Farms Wisconsin) weighing 100—200 grams each were used Potassium iodide drinking water (6×10^{-4} M) was given to all experimental animals to reduce the uptake of inorganic ^{131}I by the thyroid Animals were given checkers of Purina Labora

tory Chow (for rats) ad libitum. Two rats were housed per cage. In all experiments in which LACA was employed, a 5 % solution of LACA in the drinking water was given to the rats at least 18 hours prior to fibrinogen injection.

Walker carcinoma 256. A line of Walker carcinoma 256 (EARLE 1935, FISHER & FISHER 1961) which for some years has been carried in this laboratory and was originally obtained from Dr. Florence Millar, National Institutes of Health, Bethesda, Maryland, was transplanted by trocar into Sprague-Dawley rats. A single fragment (about 15 mg) from vascular non-necrotic tumor areas was placed subcutaneously on the right side approximately opposite the lowest rib. The transplant site had a barely palpable tumor on the seventh day after inoculation, which grew to a 10 to 14 gram tumor in six more days.

^{131}I fibrinogen injection and radioactivity measurements. Immediately prior to its injection, the previously iodinated frozen fibrinogen was thawed and diluted with fresh oxalated rat plasma to an activity concentration of about 200 μCi per ml. Diluted fibrinogen was assayed by a clottability test within 2 hours prior to injection and was found to contain 82 to 92 % clottable protein. Diluted fibrinogen solution was injected via the saphenous vein in a volume of 0.5 ml. Immediately after the injection a whole body radioactivity measurement was made by using MUTSCHLER's technique (1963, 1964). Counts were compared to those from an injection standard prepared by adding 0.5 ml of injection solution to 100 ml of distilled water made basic with several NaOH pellets in a 250 ml flask. (The standard was made basic to keep any non-protein bound ^{131}I in the reduced non-vaporizable iodide form.) Additional whole body counts were made prior to sacrifice. Immediately after sacrifice in ether, the rats were weighed. The tumor was then carefully removed, weighed, and counted. The remainder of the animal (the carcass) was similarly counted.



Fig. 1. The tumour heating apparatus

Tumor heating technique For selective tumor heating a Blue M Magni Whirl Jar Bath (Blue M Electric Company Blue Island, Illinois) was used In order to heat the tumor alone, the rat was placed in an L shaped lucite holder which had a one inch diameter hole in the outside wall of the angle through which the subcutaneous tumor could be pulled The tumor was then pulled through a small hole in a thin rubber sheet placed outside the lucite container to prevent entrance of warm water (see Fig 1)

In order to keep the rats immobile during treatment, they were anesthetized with a fluothane-oxygen anesthetic Fluothane the Ayerst Laboratories brand of halothane was kindly supplied by Dr J B Jewell of Ayerst Laboratories 685 Third Avenue New York New York 10017 Oxygen loaded with fluothane was allowed to enter the ane thesia chamber at 0 2 cubic feet per hour and 100 % oxygen entered the chamber at 2 0 cubic feet per hour

Radioautography studies For radioautographic and further histologic study tumors were fixed in a buffered 10 % formalin solution exposed to no-screen roentgen film as paraffin sections and later st ined with hematoxylin and eosin The film was exposed for 48 to 96 hours depending on the activity of the ^{131}I in the sections

Experimental results

Quantitative ev aluation of the effect of the various treatments required para meters measuring ^{131}I localization The par imeters used in this study are as follows

Percentage of injected dose per gram tumor normalized (% ID/g TN) This parameter thus involves a normalization of localized radioactivity to correct for the differences in body weights of the individual experimental rats The para meter is calculated as follows

$$\% \text{ ID/g TN} = \frac{\text{net counts per min from tumor} \times \text{whole body weight}}{\text{net counts per min from injection standard} \times \text{tumor wt}}$$

Therapeutic ratio The value of radiation treatment of a cancerous growth is usually judged according to the magnitude of the ratio

$$\frac{\text{rad absorbed by malignant tissue}}{\text{rad absorbed by normal tissue}}$$

Table 1

Effect of mechanical traumatization on tumor fibrinogen localization

Group	Number of rats	% ID/g TN	Therapeutic ratio	3 day whole body radioactivity retained (%)
No treatment	28	1 06 ± 0 07	7 9 ± 0 7	19 5 ± 1 1
Daily mechanical traumatization	[illegible]	2 36 ± 0 42*	22 2 ± 5 2*	30 4 ± 4 1*

* Indicates a statistically significant difference from the control value at the 1 % significance level
In this and following table each parameter is followed by ± standard error of the mean

This ratio can be approximated for the case of a rat bearing the Walker tumor and having been administered a certain injected dose of [131]I fibrinogen

$$\frac{\text{percentage injected dose per gram tumor tissue}}{\text{percentage injected dose per gram normal tissue}} = \frac{\%\ \text{ID/g tumor}}{\%\ \text{ID/g carcass}}$$

This latter approximate calculation will be referred to as the therapeutic ratio for the purposes of this study

Percentage of whole body radioactivity retained If one animal of an experimental group had a radioactivity retention which was greater than two standard deviation units higher than the mean of the group, it was usually an indication that some of the [131]I fibrinogen had been injected into the tissue surrounding the saphenous vein rather than intravenously and was clotted there under the action of tissue thromboplastin The data from such experimental animals was discarded The percentage of whole body retention was calculated as follows

$$\text{percentage whole body retention on day X} = \frac{\dfrac{\text{whole body counts on day X}}{\text{standard counts on day X}}}{\dfrac{\text{whole body counts at injection}}{\text{standard count at injection}}} \times 100$$

Thus, by comparison with an injection standard, the whole body counts on experimental animals were corrected for the physical decay of [131]I

Effect of epsilon aminocaproic acid (EACA) on the localization of [131]I fibrinogen in the Walker carcinoma 256 The data now reported were gathered from experiments in which control rats received either KI or EACA plus KI in their drinking water but no additional treatment In each case, Walker tumor

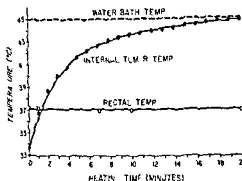


Fig 2 Effect of selective warm water tumor heating on tumor and rectal temperature

bearing rats were placed on either KI or 5 % EACA KI nine days after tumor transplant and one day prior to ^{131}I injection After three days the tumor was carefully excised The 23 control rats which had only KI in their drinking water showed a % ID/g TN of 0 44 ± 0 03 whereas this parameter for the 27 rats drinking 5 % EACA KI was 1 48 ± 0 17 Thus, an increase of tumor fibrinogen localization by a factor of 3 4 was induced by EACA This figure is within the range previously reported (BALE et coll 1962 MUTSCHLER 1963 1964)

Effect of mechanical traumatization on tumor fibrinogen localization Preliminary experiments indicated that room temperature heating of control tumors caused considerably more tumor fibrinogen localization than nontreatment of controls That this increased localization could be due to mechanical traumatization during positioning is shown by the results in Table 1

These results indicate that rough handling of the Walker tumor can lead to marked increase of ^{131}I fibrinogen localization there and that mock treatment of control animals is necessary in order that the effect of tumor heating per se can be studied

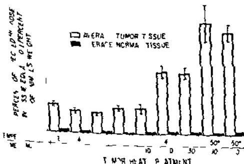


Fig 3 Effect of warm water tumor heating on localization of ^{131}I fibrinogen in Walker carcinoma 256 The percentage of injected radioactivity dose remaining in tumor and non tumor portions of rats whose tumors were subjected to heat treatment for the time and temperature indicated is plotted here in the form of a bar graph Values are expressed as percentage of injected ^{131}I dose in an amount of tissue equal to 1 of animal's total weight Vertical line at top of each bar indicates standard error of mean (Standard error of the mean values for average normal tissue were not large enough to be reproduced)

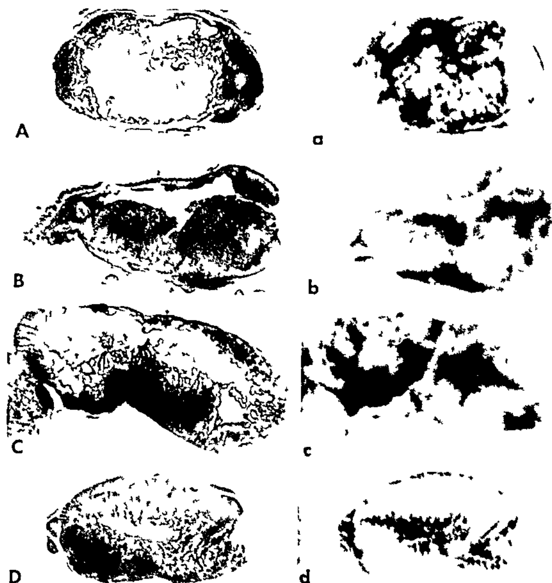


Fig 4 Photomacrographs and radioautographs from selective tumor heating study (warm water) 3 day uptake magnification × 1.4

A—a) Control tumor (KT—20) Central area of tumor section composed of light pink cytoplasmic material with low nuclear density and empty space region thought to be necrotic Outer margins of tumor (cortex) composed of cells with large dark blue nuclei and little cytoplasm Most radioactivity in transition zone between these two area

B—b) Treated tumor (42 C—20) There is a large acellular cavity just below skin surface probably filled with bloody fluid at sacrifice and surrounded by narrow band of predominately cytoplasmic cells Tumor surrounded by considerable amount of muscle and connective tissue Radioactivity in area around cavity and in connective tissue

C—c) Treated tumor (42 C—40) Tumor is considerably larger than others in the series and has a large lightly stained medulla Radioactivity in transition zone between medulla and cortex

D—d) Treated tumor (42.5 C—30) Tumor similar in structure to tumor in A—a but has a much smaller medulla Radioactivity in the lightly stained medulla

Effect of selective tumor heating on the localization of ^{131}*I fibrinogen in the Walker carcinoma 256* Actual tumor temperature during a typical heat treatment was measured by a thermistor placed in the center of the tumor with precautions taken to prevent water seepage into the tumor (Fig 2) A rapid temperature rise occurred for about 8 minutes until 43° C was reached, and then the temperature gradually increased 1 8° C in the remaining 12 minutes The water bath was kept at 45 C and the tumor temperature was monitored for 20 minutes

Results of a treatment temperature series are compiled in Fig 3 Only in the last four treatment groups was a statistically significant difference from the control values noted in fibrinogen localization

Representative stained sections and the corresponding radioautographs from each group in the above study are illustrated in Figs 4 and 5 These radioautographs suggest that ^{131}I fibrinogen localization took place in necrotic tumor tissue and that heat treatment increased tumor necrosis

At least 20 minutes at 45° C was necessary to enhance fibrinogen localization in the tumor Treatment at 50° C produced too much injury to normal tissue for it to be a feasible means of fibrinogen localization enhancement

In an additional study the hind legs of several animals were heated at 50 C 10 50° C 20' 45° C-20 and 45 C 30 The hind legs which were heated at 50 C were badly injured Hind legs which were heated at 45° C swelled somewhat at the time of treatment but did not how subsequent visible damage When the parameter % ID/g TN is compared for tumor and hind leg muscle heated at 45 C for 20 minutes one finds 3 87 ± 0 62 and 0 17 ± 0 05 for tumor and muscle respectively At this temperature then tumor tissue is much more susceptible to heat damage than is normal muscle tissue as evidenced by the degree of fibrinogen localization

Kinetics of tumor fibrinogen localization after heating at 45 C 30 Control and experimental animals were sacrificed at the following times after initiation of the 30 minute heat treatment 0 5 24 42 and 61 hours ^{131}I fibrinogen was injected intravenously just before beginning heat treatment The variation of control and experimental therapeutic ratios with time is plotted in Fig 6 This parameter of fibrinogen localization appear to increase exponentially with time in both groups after 20 hours

In the second kinetics of uptake study control and experimental rats were injected just before just after or 24 hours after treatment and sacrificed 24 hours later The results are tabulated in Table 2 The greatest 24 hour localization took place in the group injected immediately after being treated

If the two experiments are considered as a whole, several trends become

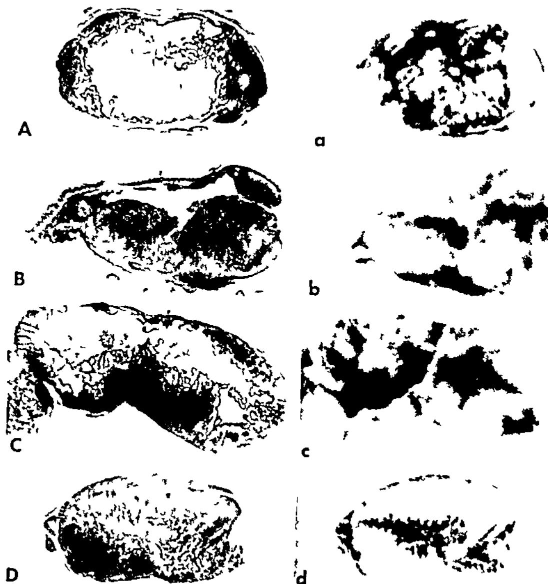


Fig 4 Photomacrographs and radioautographs from selective tumor heating study (war water) 3 day uptake magnification ×1 4

A—a) Control tumor (R T —20) Central area of tumor section composed of light pir cytoplasmic material with low nuclear density and empty space region thought to be necrot Outer margins of tumor (cortex) composed of cells with large dark blue nuclei and litt cytoplasm Most radioactivity in transition zone between these two areas

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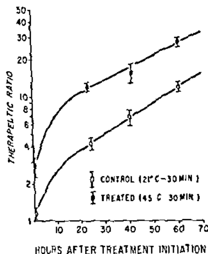


Fig 6 Kinetics of ^{131}I fibrinogen localization in Walker carcinoma 256 after warm water tumor heating at 45 C—30 Therapeutic ratio plotted against time in hours which elapsed between beginning of heat treatment and sacrifice Vertical bars at each point indicate variation allowed by the standard error of mean The therapeutic ratios of both the heated and control groups seem to increase exponentially with time after 20 hours

evident The greatest amount of radioactive fibrinogen is deposited in the tumor during the first day after treatment A smaller amount is deposited in the tumor during treatment The exponential increase of therapeutic ratio with time suggested by Fig 6 probably results from a greater biologic half time of ^{131}I fibrin(ogen) in the tumor than in the remaining body tissues (see Fig 8)

Representative stained tumor sections and the corresponding radioautographs are presented in Fig 7 There did not seem to be any change with time in the pattern of ^{131}I fixation in either the control or heated tumors In the control tumors radioactivity was found predominately in the center of the tumor in an

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Fig 5 (see opposite page) Photomacrographs and radioautographs from selective tumor heating study (warm water) 3 day uptake magnification ×1 7

E—e) Treated tumor (45 C—10) First tumor in series to show some effect of heat treatment composed almost entirely of light-staining regions Radioactivity distributed irregularly throughout this region Area of highest density composed of cells almost completely devoid of nuclei

F—f) Treated tumor (45 C—20) Most of this tumor composed of lightly stained area cells loosely packed with large nuclei and little cytoplasm surrounded by pink filaments (fibrin) Radioactivity throughout tumor and most dense in the lightly stained areas

G—g) Treated tumor (45 C—30) Tumor is similar to the one in F—f It is almost completely composed of loosely packed cells imbedded in filamentous material entire section lightly stained and radioactivity spread throughout the section

H—h) Tumor similar to both the two preceding ones Some erosion of tumor can be seen There are a small region of normal tumor cells of cortex type which has almost no radioactivity and a long blood filled cavity which is not especially radioactive Radioactivity is greatest in area immediately surrounding blood cavities and in the area bordering the cortex type cells

I— Treated tumor (50 C— 0) There is considerable tumor erosion Radioactivity is dense throughout tumor and is minimal in muscle and connective tissue

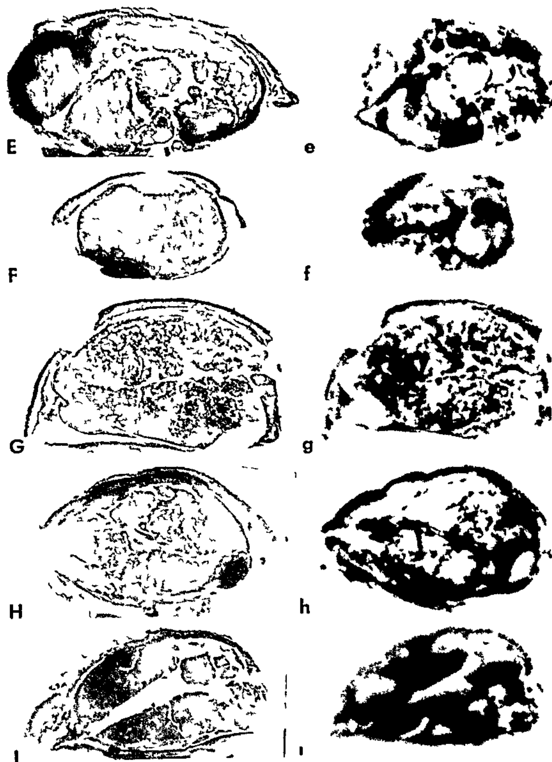


Fig 5 (for legend see opposite page)

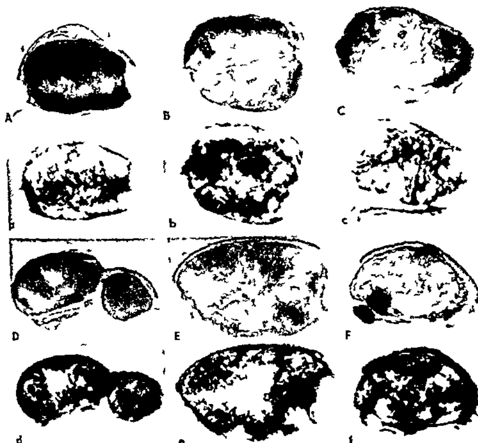


Fig 7 Photomacrographs and radioautographs from kinetic of uptake study after tumor heating at 45 C—30 Magnification ×1 1

A—a) B—b) and C—c) Control tumors (R T —30) 1 2 and 3 days The light pink staining medullary area expands to occupy more of tumor as time passes Radioactivity located predominately in this light medullary area and in transition zone immediately surrounding it Radioactivity becomes more widespread with time as it follows the light pink stained area

D—d) E—e) and F—f) Treated tumors (45 C—30) 1 2 and 3 days Radioactivity widespread throughout tumor on first day after treatment only a small area of medullary tissue is low in radioactivity At one day after treatment (D—d) the cortex and medulla are not clearly demarcated and whole tumor stains pinkish blue Cellular structure fairly regular with polygonal tumor cells having moderately large nuclei interspersed in pink fibrinous material On second and third days after treatment (E—e and F—f) two definite zones appear Radioactivity is localized mainly in the light pink staining regions characteristic of the medulla and is characteristically absent from the dark blue staining areas that might represent tissue not affected by heat treatment since they usually lie on the side of the tumor which was next to the body

Table 2

Kinetics of tumor fibrinogen localization after heating at 45° C—30′ — 24 hour uptakes starting at various times in relation to treatment

Treatment	Number of rats	%ID/gTN	Therapeutic ratio	Whole body radioactivity retained (%)	Average rat weight gram	Average tumor weight gram
Control RT—30 injected prior to treatment	8	1.77 ± 0.29	4.2 ± 0.5	51.1 ± 3.9	150 ± 7	5.55 ± 0.50
Control injected following treatment	5	1.99 ± 0.08	4.0 ± 0.2	59.0 ± 1.3	148 ± 4	2.12 ± 0.33
Control injected 24 hours following treatment	5	1.87 ± 0.23	4.7 ± 0.6	45.1 ± 1.8	154 ± 3	2.06 ± 0.55
45° C—30′ injected prior to treatment	7	4.34 ± 0.48	12.0 ± 0.8	53.6 ± 2.4	147 ± 7	5.09 ± 1.33
45° C—30′ injected following treatment	5	6.94 ± 1.11	12.8 ± 1.7	67.5 ± 3.9	151 ± 3	3.38 ± 0.61
45° C—30′ injected 24 hours following treatment	5	1.65 ± 0.29	3.8 ± 0.8	50.1 ± 1.0	145 ± 5	2.21 ± 0.98

area thought to be necrotic and which stained pink in hematoxylin and eosin. In the heated tumors, radioactivity was also found in areas staining pink in hematoxylin and eosin but this area occupied a greater portion of the tumor and usually included the tumor cortex. Microscopic examination of sections of heated tumors and their radioautographs suggests that ^{131}I radioactivity is fixed in a lightly pink stained filamentous network located outside blood capillaries and sinuses. It is difficult, however, to pin point the source of radioactivity at a cellular level with the radioautograph procedure here employed.

^{131}I albumin localization in the heated Walker carcinoma. Nine days after Walker tumor transplant, 29 rats were given 5 % EACA-KI as drinking water. On the tenth day after transplant, each rat was given about 100 μCi of ^{131}I albumin intravenously. As soon thereafter as possible, the tumors of 14 experimental animals were treated in 45° C water for 30 minutes as previously described. Tu

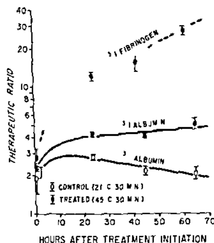


Fig 9 Comparison of ¹³¹I fibrinogen and ¹³¹I albumin therapeutic ratios versus time after tumor heating

treatment was such that the internal tumor temperature reached approximately 44° C and was maintained at that or greater temperatures for several minutes It is possible that this temperature range must be reached before bradykinin or some other mediator of increased capillary permeability is released in the heated tumor tissue

Although treatment for 10 or 20 minutes at 50 C caused the most marked increase in tumor fibrinogen localization encountered in the warm water study this treatment temperature caused considerable injury to normal tissue and was consequently excluded from further investigation

In general control tumors exhibited a necrotic region in their center surrounded by a zone in which the cells were starting to undergo necrosis presumably because of an insufficient blood supply Around this region the tumor cortex composed of viable tumor cells and stroma was situated The radioactivity in control tumors was usually located at interfaces between tumor and normal tissue and in the zone between the viable tumor cortex and the necrotic tumor core It seems reasonable that materials released from dying cells induced an inflammatory reaction in the neighboring regions and thus blood borne ¹³¹I fibrinogen was deposited there When heat was applied to tumor tissue the metabolic demands of the viable tissues were increased and their blood supply already being under considerable stress was insufficient for the demand and the cells died In such regions cellular materials released by autolysis could increase the low grade inflammatory reaction occurring and induce extravasation and clotting of blood borne fibrinogen Thus an uneven distribution of ¹³¹I deposition would be expected and would depend on how close to being overstressed the

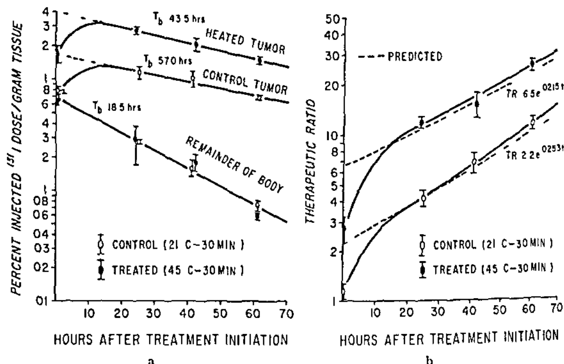


FIG 8 Specific ^{131}I fibrinogen activity of tumor and carcass versus time after heating in 45 C water (a) and therapeutic ratio versus time after treatment (b)

mors of 15 control animals were treated for the same length of time but with the water bath at 21° C Immediately after this treatment, two experimental and three control rats were sacrificed and the previously described parameters were determined At 24, 45 and 65 hours after treatment initiation, four experimental and four control rats were sacrificed and the usual parameters were calculated The results of this study are plotted in Figs 9 and 10

Discussion

The stated object of this series has been to investigate a variety of methods by which the localization of radioiodinated fibrinogen in the Walker carcinoma 256 might be enhanced Results obtained in the course of this investigation suggest that selective heating of a Walker tumor markedly increases the localization of previously injected ^{131}I fibrinogen in the tumor The tumor had to be exposed to an environment of about 45° C for at least 20 minutes to obtain significantly greater localization of ^{131}I fibrinogen within the tumor

It is tempting to apply the findings of ROCHA E SILVA (1963) to the problem of tumor fibrinogen localization after heat treatment No significant increase in tumor fibrinogen localization above the control level was noted until heat

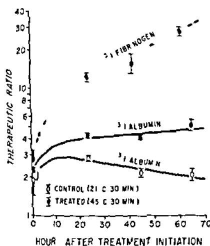


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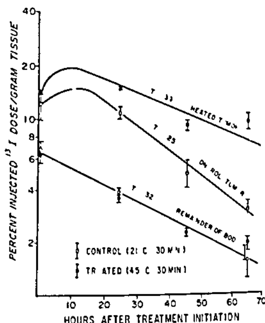


Fig 10 Specific activity of ^{131}I albumin in tumor and carcass versus time after heating in 45 C water (conditions as in fig 8)

circulation of particular tumor areas was Radioautographs of heated tumors evidenced a radioactivity deposition which became more widely distributed as the amount of heat treatment was increased

A theoretical analysis of tumor fibrinogen localization after treatment in 45° C water for 30 minutes is presented in Fig 8 On the left of the figure, the logarithms of percentage of injected dose per gram (% ID/g) of heated and control tumor tissue and for the carcasses of heated and control rats are plotted against time These curves are consistent with the hypothesis that ^{131}I fibrinogen is deposited in the tumor primarily during the first 20 hours after injection whether the tumor is heated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time Because the % ID/g data contain no component of ^{131}I physical decay, the exponential decrease of tumor radioactivity concentration with time indicates a removal of ^{131}I from the tumor at a rate proportional to the amount present

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor The carcass radioactivity concentration is approximately the same initially in the treated and control groups and the carcass biologic half life of ^{131}I fibrinogen is equal in the treated and control groups

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation $A_t = A_0 e^{-\lambda t}$ where A_t is tissue radioactivity concentration, % ID/g at any time t For the tumors, the

relation holds when t is greater than about 20 hours A_0 is the hypothetical initial tissue radioactivity concentration, % ID/g λ is the biological decay constant in hours[-1] and is equal to 0 693/T_b where T_b is the biologic half life in hours of ^{131}I fibrinogen in the tissue in question The time in hours after treatment initiation is represented by t

When the suitable constants determined in Fig 6 are substituted into the above relation the following mathematical descriptions for tissue radioactivity versus time are obtained

treated tumor	$A_t = 4\ 0e^{-0159t}$	(a)
control tumor	$A_t = 1\ 5e^{-01\ t}$	(b)
treated carcass	$A_t = 0\ 62e^{-375t}$	(c)
control carcass	$A_t = 0\ 68e^{-375t}$	(d)

The therapeutic ratio as a function of time for control and treated groups can be calculated from relations b d and a c, respectively

$$\text{T R control} = \frac{A_t\ (\text{control tumor})}{A_t\ (\text{control carcass})} = 2\ 2t^{0\ 53t}$$

$$\text{T R treated} = \frac{A_t\ (\text{treated tumor})}{A_t\ (\text{treated carcass})} = 6\ 5t^{0\ 15t}$$

These calculated theoretical expressions for the therapeutic rations are plotted as functions of time on the right side of Fig 8 The variations of the experimental therapeutic ratios with time are also plotted here as they were in Fig 6 The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference of biologic half life of ^{131}I fibrinogen in the tumor and carcass

In order to assess the effect of the warm water treatment on the average total radiation doses to tumor and carcass the concept of effective half life must be included

$$T_{\text{effective}} = \frac{T_b \times T_p}{T_b + T_p}$$

where T_b = biologic half life (just determined), T_p = physical half life (192 hours for ^{131}I)

since $D_\beta(\infty) = \text{constant} \times C_0 \times T_{\text{eff}}$ (adapted from LOEVINGER et coll)

where $D_\beta\ (\infty)$ is the average total radiation dose from ^{131}I β s absorbed by a tissue in which the initial radioactivity concentration is C_0 and the effective half life of ^{131}I is T_{eff} The ratio of total tumor dose to total carcass dose is thus

$$\frac{D_{\beta}(\infty)\ \text{tumor}}{D_{\beta}(\infty)\ \text{carcass}} = \frac{C_0 \times T_{\text{eff}}\ (\text{for tumor})}{C_0 \times T_{\text{eff}}\ (\text{for carcass})} = T R_0 \times \frac{T_{\text{eff(tumor)}}}{T_{\text{eff(carcass)}}}$$

$$\frac{\text{average } \beta \text{ dose to tumor}}{\text{average } \beta \text{ dose to carcass}} = \begin{matrix} 6\ 5 \times 2\ 1 = 13\ 7 \text{ for treated group} \\ 2\ 2 \times 2\ 6 = \ 5\ 7 \text{ for control group} \end{matrix}$$

This theoretical calculation indicates that the warm water treatment improved the ^{131}I fibrinogen radiation therapy of the Walker tumor nearly 250 per cent

Preliminary experiments by BALE et coll (1964) have indicated that heating tail transplanted Walker tumors at 41 5° C for five hours markedly increases tumor localization of ^{131}I labeled antifibrin antibody

Several conclusions can be drawn from ^{131}I albumin results The therapeutic ratio for fibrinogen injected animals (Fig 9) is nearly six times greater three days after treatment than that for animals given ^{131}I albumin and identical heat treatment Data presented in Fig 10 suggest two reasons for this difference in therapeutic ratios Although the biologic half lives of ^{131}I albumin and ^{131}I fibrinogen are approximately the same in the heated Walker tumor, the maximum tumor radioactivity concentration is nearly two times greater after ^{131}I fibrinogen administration In addition, the biologic half life of radioalbumin in the non tumor portion of the rat s body is almost twice as long as that of radio fibrinogen These factors combine to produce considerably less increase of therapeutic ratio with time in the ^{131}I albumin injected rats

This study seems to indicate that proteins such as fibrinogen and antifibrin antibody whose tumor localization is associated with the clotting mechanism, are unusual in their susceptibility to be retained in the heated Walker tumor without marked concurrent retention in the non tumor portions of the host

Acknowledgement

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SUMMARY

Heating of the Walker carcinoma 256 by immersion in a water bath at 45 C for 30 minutes led to a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which localized in the tumor Such heat treatment increased the tumor radiation therapy dose from ^{131}I almost 250 per cent Heated muscle in the neighborhood of tumor did not localize ^{131}I fibrinogen Radioactivity deposition coincided with histologically demonstrable areas of tumor damage ^{131}I albumin was not effectively cleared from non tumor tissues

ZUSAMMENFASSUNG

Eine Erwarmung des Walker Carcinoms 256 durch Eintauchen in ein Wasserbad von 45 C 30 Minuten lang fuhrt zu einem kraftigen Anstieg der Menge intravenos injizierten 131I Fibrinogens die sich im Tumor ansammelt Eine derartige Warmebehandlung steigert die therapeutische Strahlendosis des Tumors durch 131I um beinahe 250 % Die erwarmte Muskulatur der Umgebung des Tumors nimmt nicht 131I Fibrinogen auf Die Ablagerung der Radioaktivitat stimmt mit der histologi ch nachweisbaren Ausbreitung der Tumor schadigung uberein 131I Albumin wurde nicht effektiv von den nicht Tumor Geweben befreit

RESUMÉ

L elevation de temperature dans un carcinome 256 de Walker par immersion dans un bain d eau a 45 C pendant 30 minutes augmente de façon importante la quantite de fibrinogene marque par 131I injecte par voie intraveineuse qui se localise dans la tumeur Ce traitement thermique augmente presque de 250 pour cent la do e d irradiation fournie par 131I le muscle chauffe dans le voisinage de la tumeur ne fixant pas le fibrinogene 131I La fixation de la radio-activite coincide avec des dommages à la tumeur mis en evidenc par l histologie L albumine marquee par 131I n est pas deplacee hors des tissus no i tu moraux par ce traitement thermique

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CELLULAR AUTOIMMUNE REACTIONS FOLLOWING RADIOIODINE TREATMENT FOR HYPERTHYROIDISM

by

NINA EINHORN J WASSERMAN and T PACKALEN

Radioiodine therapy is known to be followed by an increase in the circulating thyroid antibodies (BUCHANAN et coll 1962 IRVINE 1964 O GORMAN et coll 1964 EINHORN et coll 1965) In hyperthyroidism there may be an increase in antibodies to thyroid cytoplasmic antigen, though not to thyroglobulin (EINHORN et coll 1965 JONSSON et coll 1968) and in euthyroidism an increase in antibodies to both thyroid cytoplasmic antigen and thyroglobulin (EINHORN et coll 1966) This increase in the circulating antibodies is temporary and lasts abou a year after ^{131}I therapy (EINHORN et coll 1965) A significantly highe frequency of positive serologic reactions has occurred in patients developin hypothyroidism within one year of ^{131}I treatment for hyperthyroidism (EINHOR et coll 1965)

It is generally believed that the specific tissue damage occurring in th autoimmune states is mediated by cellular reactivity It would therefore be o interest to know whether patients treated with radioiodine develop cellular r activity to thyroid antigens, as happens in autoimmune states This knowledg

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might provide information on whether the autoimmune reaction induced by the local irradiation is of significance for the effect of radiation The response of lymphocytes from such patients to thyroglobulin has been studied in vitro

Thyroglobulin has been preferred to the microsome antigens in order to keep allotypic differences to a minimum The stimulation of lymphocytes in vitro in the presence of antigen seems not to be closely dependent on circulating antibodies although in some way related to the state of delayed hypersensitivity (MILLS 1966, OPPENHEIM et coll 1967) No definite proof has been presented, however, that such stimulation is a manifestation of delayed hypersensitivity

During recent years several methods of examining lymphocytic reactions to antigens in vitro have been developed One method relies on the fact that an increase in the incorporation of isotope labelled thymidine by the cells indicates an increase in DNA synthesis in the presence of antigen (LING 1968) This method has been used in the experiments now reported The stimulation of DNA synthesis by PPD tuberculin was tested on lymphocytes from subjects with positive skin reactivity to turberculin to verify that the method was adequate when an active stimulant was used Lymphocytes and the thyroid glands of guinea pigs immunized with human thyroglobulin in complete adjuvant were studied in addition to lymphocytes from patients treated with radioiodine

Material and Methods

Human lymphocyte donors The material consisted of 25 subjects divided into three groups comprising 11 healthy controls, 8 patients who had previously received radioiodine therapy and 6 patients with subacute thyroiditis

The patients given radioiodine were all selected as having circulating antibodies to thyroglobulin or thyroid cytoplasmic antibodies at the time of examination Their passive haemagglutination titres ranged from 1/80 to 1/12 500, four of them also had antibodies to cytoplasmic antigen as assessed by the fluorescent antibody (FA) technique Seven of the patients were women and one was a man, their ages ranged from 49 to 69 years They were examined 1—4 years after radioiodine treatment for hyperthyroidism, four of them having diffuse and four nodular goitres The total dose of radioiodine ranged from 3 5 to 33 mCi ^{131}I

All the six patients with clinical signs of subacute thyroiditis had antibodies (FA) to microsome antigen, and four of them had haemagglutinating antibodies to thyroglobulin in titres ranging from 1/400 to 1/4 000 000 The five women and one man were from 29 to 43 years of age

Eleven subjects, nine women and two men, aged between 24 and 48 years

were taken as controls none had demonstrable humoral antibodies to thyroid antigens

Experimental animals Nine male guinea pigs weighing about 400 g were immunized by injecting 2 mg of human thyroglobulin in complete Freund s adjuvant as described for the immunization of this species with guinea pig thyroglobulin (WASSERMAN & PACKALEN 1965) After 3 weeks the skin hyper sensitivity to thyroglobulin was examined and the animals killed, the thyroid glands then being examined for histologic changes and the lymphocytes for stimulation of the DNA synthesis in the presence of thyroglobulin Untreated male guinea pigs were used as controls

Methods Thyroglobulin was prepared from human thyroid tissue removed during surgery by the method described by LINDER & WEIBULL (1960) Several batches each from one single gland, were used PPD tuberculin (purified protein derivative) (kindly supplied by Parke Davis & Co Detroit Michigan) without preservatives was employed One hundred milligrammes of phytohaemagglutinin were dissolved in 5 ml Hank Tris buffer and frozen in small portions About 50 to 100 ml of blood were withdrawn from a cubital vein of the patients and controls and defibrinated by cautious agitation with glass beads The lymphocytes were isolated from the defibrinated blood by the method of COULSON & CHALMERS (1964) The cells were washed twice in Hank Tris buffer, counted in a Burker chamber and suspended in Eagle s medium supplemented with 10 % heat inactivated autologous serum or fetal calf serum One million lymphocytes were pipetted into conical tissue culture tubes The antigens in appropriate concentrations were added to the cells in a volume of 0 5 ml or 250 to 500 mcg of PHA M in a volume of 0 5 ml

Tubes without antigens served as controls The total volume of the incubation mixtures was 1 0 ml The tubes were loosely closed with screw caps and incubated with a continuous flow of a mixture of 5 % carbon dioxide in air After 72 hours 0 4 μCi of ^{14}C thymidine (specific activity 35 7 mCi/mM or 54 4 mCi/mM) was added to all tubes After a further 24 hours the tubes were cooled in ice water and washed 4 times in cooled buffer One drop of saturated ^{12}C thymidine was added to the first two washings The washed cells were dissolved in 1 0 ml of concentrated formic acid and the liquid was transferred to planchets After drying the radioactivity was determined in a gasflow counter equipped with a Geiger Muller tube The uptake of ^{14}C thymidine in each sample was expressed in counts per minute and the means of the values for duplicate tubes were taken The results were expressed as the ratio between the counts per minute obtained with and without antigen This ratio was called the lymphocyte stimulation index (LSI)

Spleen cells were used in the guinea pig experiments in place of blood lymphocytes The spleen was removed aseptically and cut into small pieces

Suspensions of spleen cells were prepared by mincing the tissue in culture medium In every other respect the method was the same as that used in the experiments with human blood lymphocytes The stimulation tests were occasionally performed twice, with either autologous serum or fetal calf serum added to the culture medium The results were the same for the two media

When thyroglobulin was used as the antigen, 10, 100, 1 000 or 5 000 mcg were added to each tube in eight subjects, three of these concentrations of antigen were used *in an additional thirteen subjects In fourteen of these subjects* the highest stimulation of the DNA synthesis was observed in the tubes to which 1 000 mcg thyroglobulin had been added, in three in the tubes to wich 100 mcg and in four subjects in the tubes to which 10 mcg had been added The stimulation was never better with 5 000 than with 1 000 mcg, only one preparation with 1 000 mcg of thyroglobulin per tube was therefore tested With PPD as antigen 5 mcg were added per tube

Tubes with PHA M were *included as positive controls in each experiment* The phytohaemagglutinin always strongly stimulated DNA synthesis as measured by the uptake of ^{14}C thymidine

Humoral antibodies to thyroglobulin and thyroid cytoplasmic antigens were tested at the National Bacteriologic Laboratory (JONSSON et coll 1968)

The time after treatment was counted from the last radioiodine dose in patients given more than one dose of radioiodine

Results

The mean of the lymphocyte stimulation index in the healthy controls was 1 04 ± 0 11, in the patients receiving radioiodine therapy 1 45 ± 0 25 and in the patients with subacute thyroiditis it was 1 50 ± 0 23, the differences between these groups were not statistically significant However, in the presence of *thyroglobulin* the lymphocyte stimulation index ranged in healthy donors from 0 62 to 1 57 (Table 1), and in the radioiodine treated patients from 0 70 to 3 00 Three of the eight patients had an index higher than that for any of the eleven healthy controls (Ht3, Ht4, Ht5 Table 2)

The index ranged from 0 90 to 2 30 in subacute thyroiditis and in two of the six patients the value was higher than for any of the eleven healthy controls (T4 and T6, Table 3)

No correlation between the values of the lymphocyte stimulation index in the presence of thyroglobulin and of humoral antibodies to thyroglobulin, as tested by passive haemagglutination of tanned cells could be found

Table 1

Lymphocyte stimulation index (LSI) in the healthy controls — Mean of LSI = 1.04 ± 0.11 (standard error of the mean)

Subjects	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
LSI	1.57	1.37	0.39	1.09	0.97	1.40	0.62	0.86	0.71	1.41	1.05

Table 2

Lymphocyte stimulation index (LSI) in the patients treated with ¹³¹I for hyperthyroidism — Mean of LSI = 1.43±0.25 (standard error of the mean)

Subjects	Ht1	Ht2	Ht3	Ht4	Ht5	Ht6	Ht7	Ht8
LSI	1.14	1.09	1.63	1.69	3.00	1.30	0.70	0.90
Years after radiotherapy	1	3	1	3	4	3	2	3
Total ¹³¹I dose mCi	33	22	12	3.5	27	6	5	3.5

Table 3

Lymphocyte stimulation index (LSI) in Hashimoto thyroiditis — Mean of LSI = 1.50±0.23 (standard error of the mean)

Subjects	T1	T2	T3	T4	T5	T6
LSI	1.40	0.95	1.43	2.04	0.90	2.30

The addition of PPD tuberculin stimulated DNA synthesis in both lymphocyte donors with a lymphocyte stimulation index of 4.9 and 7.0 these controls gave positive skin tests to tuberculin in a dilution of 1:1 000

When the human thyroglobulin used in the above stimulation study was injected with complete Freund's adjuvant into the 9 guinea pigs a delayed type skin sensitivity to thyroglobulin developed Stimulation of DNA synthesis in the presence of thyroglobulin was demonstrated in lymphocytes from most of the animals morphologic signs of thyroiditis were observed in the four submitted to histologic examination

Discussion

It is evident from the stimulation of DNA synthesis by PPD tuberculin that the experimental system employed in this study was effective when the stimulant was active

Immunization of the guinea pigs with human thyroglobulin in complete adjuvant produced the usual sequence of events (McMaster et coll 1961, Wasserman & Packalen 1965), of particular interest was the development of thyroiditis Delayed hypersensitivity is a specific immunologic manifestation induced by many heterologous antigens incorporated in complete adjuvant The induction of thyroiditis points to an organ specific activity of the thyroglobulin preparation used

The results of the experiment with lymphocytes from patients with thyroiditis are in agreement with those of other workers Only occasional positive results have been reported (Lycette & Pearmain 1965, Forbes 1965) De Groot & Jaksina (1969) in Hashimoto thyroiditis observed no stimulation of the thymidine incorporation in lymphocytes neither by thyroid supernatant fraction nor by thyroid microsomal fraction

The main object of the present experiments was to ascertain whether lymphocytes from patients treated with radioiodine and displaying circulating antibodies to thyroid antigens were sensitized to thyroglobulin The groups composing the material are small and the results preliminary However, three of the eight patients receiving radioiodine therapy for hyperthyroidism had a higher lymphocyte stimulation index than any of the eleven healthy controls, and one recorded a value higher than the mean for the control group ± 5 times the standard error This increase in the stimulation of blood lymphocytes to thyroglobulin in some of the radioiodine treated patients may either be due to the effect of this local radiotherapy to the thyroid gland or may have been present before the radioiodine had been given A considerable proportion of untreated patients with hyperthyroidism have demonstrable antibodies to thyroglobulin and to cytoplasmic antigens, no information is, however, available on the lymphocyte stimulation index in untreated hyperthyroidism (De Groot & Jaksina 1969)

It has previously been reported (Einhorn et coll 1965, Jonsson et coll 1968) that the increase in circulating thyroid antibodies after radioiodine therapy for hyperthyroidism is confined to antibodies to thyroid cytoplasmic antigens and is temporary, lasting about a year There is no information as to whether the cellular reactivity in this respect follows the serologic reactivity All the patients in this series had been treated with radioiodine more than a year previously

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SUMMARY

A material of 25 subjects including eight patients who had previously received radio iodine therapy was examined to determine whether cellular reactivity to thyroid antigens developed The results which are preliminary are discussed in detail

ZUSAMMENFASSUNG

An einem Material von 25 Individuen einschliesslich acht Patienten die vorher mit radioaktivem Jod behandelt worden waren wurden Untersuchungen angestellt um zu ermitteln ob Zeichen einer zellularen Reaktion gegen Schilddrusenantigene gefunden werden konnten Die Resultate die preliminar sind werden diskutiert

RÉSUMÉ

Les auteurs ont cherche s'il apparait un reactivite cellulaire aux antigenes thyroidiens chez 25 sujet dont huit malades les avaient éte traites par l'iode radio-actif pour hyperthyroid Ils examinent en detail les resultats qui sont préliminaires

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APPLICATORS FOR REMOTE AFTERLOADING TECHNIQUE FOR OPTIMUM PELVIC DOSE DISTRIBUTION IN CARCINOMA OF THE UTERINE CERVIX

by

INGEMAR JOELSSON and ANDERS BACKSTROM

Attempts at reaching optimum dose distribution in the pelvis from intra cavitary radiation for carcinoma of the uterine cervix have interested the clinician for many years and a wealth of literature is available on the subject Tradition ally an improved dose distribution is aimed at by an increase in the source surface distance an elongation of the intra uterine source and by using multiple radia tion sources preferably spread out in the superior part of the vagina Lack of space often imposes limitations and difficulties are encountered regarding the maintenance of the position of the sources during the treatment time both in their interrelationship and to the anatomic configurations of the patient

The design by NEARY (1947) of a combined intra uterine and intravaginal high activity irradiator employing specific screens in the direction of the bladder and the rectum represents one attempt to achieve improvement in the dose at

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Table 1

Physical characteristics of various materials for the attenuation of radiation from ^{137}Cs

	Z number	Atomic weight	Density	First HVL in mm (Cs)
anium	92	238	18 8	2 9*
ngsten	74	184	18 6—19 3	4 0 and **
ad	82	207	11 0	5 5
tinum	78	195	21 4	3 2 **
old (pure)	79	197	19 3	3 6* *
old (18 carat)	71***		16 7	4 2* *

According to THORAEUS 1965

** Tungsten alloy density 17 7

Calculated from values in GRODSTEIN 1957

* * Calculated in accordance with MAYNEORD 1937

een stepwise increased from the previously used content of 120 to 140 mg radium to three times this amount The vaginal applicator was loaded with a cesium source train of 600 mCi activity Distribution of the sources at lateral parts of the ring together with tungsten absorbers at selected locations resulted in a dose reduction in the direction of the urinary bladder and the rectum A radium source train of variable activity (100 to 250 mg Ra) was used for the intra uterine sound this applicator combination will be referred to as system No 1 The recently constructed vaginal applicator model utilizing additional shielding material was employed together with an intra uterine steel sound both tubes being equipped with cesium source trains The activity of the sources in each applicator was 600 mCi This applicator combination will be called system No 2

In the design of the vaginal applicator of the No 2 system various high Z materials were considered (Table 1) Uranium was disregarded because of its high reactivity Lead had a too high HVL figure and platinum was too costly Tungsten manufactured in sintered form and used as an alloy with copper and nickel was unsuitable because of difficulties encountered in machining it Eighteen carat gold which has a high resistance to corrosion and is easy to machine was therefore chosen

The vaginal applicator of the No 2 system was intended to be so designed as to reduce the doses in the ventral and dorsal directions thereby shielding the bladder base and the anterior wall of the rectum It was decided not to change the arrangement of sources and absorbers from the previous design by WALSTAM It had been found however in a study in 4 patients with the

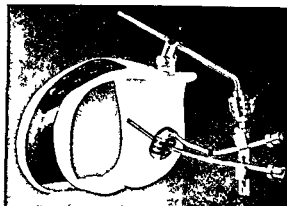


Fig 1 The intracavitary applicators of the remote afterloading unit are held in place by a fiberglas corset

the pelvic wall More recently proposals have suggested the incorporation of specific shields of high Z metal in the applicators of the current Stockholm method, in combination with the use of medium or low energy gamma radiation sources (KOTTMEIER & WALSTAM 1962) The introduction of the remote afterloading technique opened further prospects WALSTAM (1965) utilized lateral placement of ^{137}Cs sources surrounded by tungsten absorbers in an early version of an afterloaded vaginal applicator in order to extend the dose distribution laterally The vaginal applicator was used, however, together with an intra uterine steel sound loaded with radium, it was anticipated that the resulting dose distribution in the pelvis would not differ considerably from that of the current Stockholm technique Development of an applicator with increased shielding particularly of the bladder and rectum, has therefore continued One of these applicator models has been introduced into the routine clinical work after determination of the dose distribution in a phantom The relationship between the doses at the pelvic wall and the dose at the posterior part of the bladder and the anterior wall of the rectum has been established in a series of patients

Intracavitary applicators With the Stockholm afterloading technique the intracavitary applicators are attached to the patient by means of a special corset (Fig 1) This arrangement decreases the risk of tissue damage due to pressure even if the applicator is heavy In addition, the gamma radiation from ^{137}Cs is of fairly low energy i e 0 66 MeV, which reduces the necessary thickness of the material used for shielding purposes The bar and ball joint system also allows the final adjustment of applicator site to be made after the patient is released from the lithotomy position

Two separate sets of intracavitary applicators were used, one of these consisted of the ring shaped vaginal applicator described by WALSTAM (1965) together with an intra uterine stainless steel sound The source activity for this set has

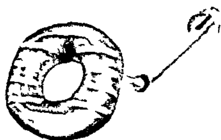


Fig 2 The vaginal applicator of the remote afterloading unit The gold screens and flanges are covered with araldite

plicators The accuracy of this determination was ± 8 % (standard deviation as percentage of the mean)

It was observed that in a section through the ring the 25 rad/h isodose rate was 78 mm lateral from the center but only 35 mm ventral and dorsal from the center (Fig 3 upper diagram) Ten millimeters ventral or dorsal from the outer surface of the ring the dose rate was less than 25 rad/h while at the same distance lateral from the surface of the ring the dose rate was 125 rad/h The factor of dose reduction in the ventral and dorsal directions was between 5 and 6

The 25 rad/h isodose curve was 35 mm ventrally or dorsally to the center of the applicator and 64 mm cranially or caudally to the center in a section perpendicular to the ring (through the shaft of the vaginal applicator) Ten millimeters ventrally from the outer surface the isodose rate was still less than 25 rad/h while 10 mm cranially to the outer surface of the ring the isodose rate was about 150 rad/h In this plane the factor of dose reduction in the direction of the bladder and the rectum was approximately 6 (see Fig 3 lower diagram)

In clinical practice the vaginal applicator is used in combination with the uterine stainless steel sound which has an outer diameter of 8 mm and a wall thickness of 1 mm The intra uterine activity (600 mCi ^{137}Cs) is distributed over a length of 58 mm 6 cesium sources each measuring 8 mm × 3 mm separated into three sections by two spacers each measuring 5 mm × 3 mm Uterine steel sounds are available in lengths between 7 and 9 cm measured from the 35 bend (always placed in the center of the vaginal sound) to the distal end The dose distribution around the combined vaginal and intra uterine applicators was measured in the section perpendicular to the ring through the center of the uterine sound The dose rates along the bisectrix of the anterior angle between the vaginal ring and the uterine sound were 600 400 and about 200 rad/h at 10 20 and 40 mm respectively, with corresponding figures posteriorly of 700 450 and 200 rad/h (Fig 4 upper diagram) The 50 rad/h isodose rate curve was

Table 2

Doses in rad (mean values and limits of observations in 4 patients) in three parts of 19 mm length and in one part of 6 mm length (under the inguinal ligament) of the right iliac vein during intracavitary radiation treatment with the remote afterloading technique applicator system No 1

	Parts of iliac vein			Under inguinal ligament
	Cranial	Middle	Caudal	
Limits	250—370	336—430	213—410	230—302
Mean	314	400	357	277

intracavitary applicator combination No 1 that the radiation doses in the right common and external iliac veins, determined by thermoluminescence dosimetry, reached a maximum of 400 rad during one course of treatment (Table 2) The mean dose during the same treatment on the posterior wall of the bladder was 2 500 rad and on the anterior wall of the rectum 2 000 rad The relationship is the same as for the current Stockholm technique Additional gold shielding was therefore applied to the anterior and posterior parts of the ring of the vaginal applicator of the No 2 system The gold shielding was shaped into the form of a part collar around the stainless steel sound to produce a sufficiently wide zone of protection Care was taken to adjust the gold shield as close to the radiation sources as possible in order to increase the space angle it occupied Discs of gold were also attached to the periphery of the steel sound between each radiation source The sizes of the discs were chosen to allow only narrow beams of radiation to reach the surrounding regions Thus in the lateral direction close to the applicator, a reduced surface dose was obtained since small tissue volumes received radiation from only one radiation source At greater distance from the ring arbitrarily chosen tissue volumes were found to receive irradiation from all three radiation sources

The vaginal applicator with the gold shields was covered with araldite The total diameter of the applicator was 54 mm and the outer diameter of the stainless steel tube was 8 mm (Fig 2)

The isodose curves around the vaginal applicator were determined by means of an automatic isodose recorder (LARSSON et coll 1963) with some modifications The instrument was equipped with an anthracene crystal placed inside an aluminium tube connected to a photomultiplier The dependence of the dose response on radiation quality for this crystal was found to be within ± 5 % in the actual photon energy range The dose rates were measured with thermoluminescence detectors placed in a perspex fixture firmly attached to the ap

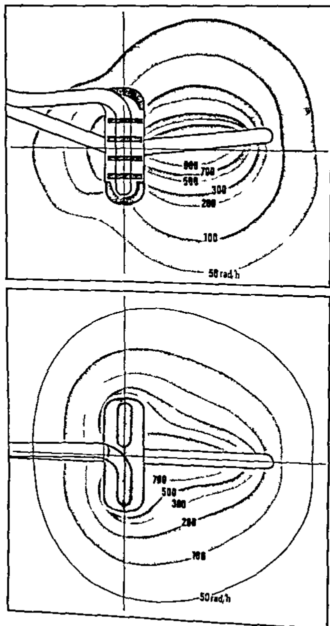


Fig 4 Dose distribution in the sagittal section through the center of the intra uterine sound (upper diagram) and in a frontal oblique plane through the intra uterine applicator (lower diagram) of the combined applicator of the No 2 system.

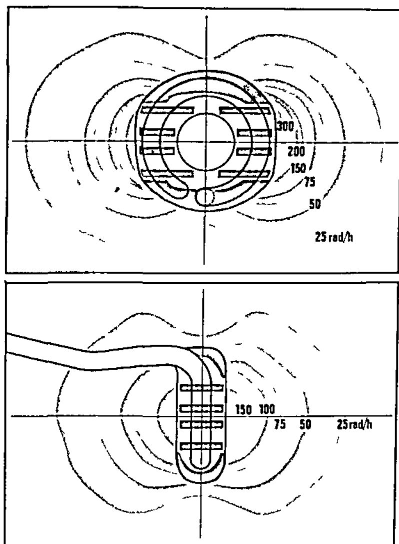


Fig 3 Dose distribution in the plane of the ring of the vaginal applicator (upper diagram) and in a plane perpendicular to the ring of the vaginal applicator (lower diagram) of the No 2 system

45 mm lateral to the outer surface of the vaginal applicator in the section through the intra uterine steel sound which forms an oblique angle with the ring, at a level 20 mm cranial to the external os 700, 310, 200 and 100 rad/h dose rates were recorded 10, 20, 30 and 50 mm, respectively lateral to the center of the intra uterine sound (Fig 4 lower diagram)

Methods Eight patients with I b or II a lesions were subjected to lymphography, phlebography and bilateral placement of thermoluminescence radiation detectors in accordance with techniques described in detail elsewhere (JOELSSON

occurred between the dose rates at the anterior wall of the rectum measured in lithotomy and supine positions (Table 3) The range of differences was large with a minimum of — 30 % and a maximum of + 94 % with a mean of + 18 % Six of the 18 patients exhibited differences of over + 20 % and for 4 of them the difference amounted to more than + 40 % The mean difference of + 18 % cannot be explained by inaccuracies in measurements but sugges s a true difference between dose rates in the two positions no such differences were observed in dose rates on the posterior wall of the urinary bladder For the detailed description of the procedure of dose rate measurement the reader is referred to a previous paper by JOELSSON & BACKSTROM (1969)

Results

The mean values of the doses over one course of intracavitary treatment were 2 900 rad at the base of the bladder and 2 000 rad at the anterior wall of the rectum The Siemens Gammameter is 6 % more sensitive for radium than for cesium within 30 mm of the source and the values were correspondingly corrected correction for decreased sensitivity of the instrument with an increase in temperature was also attempted (JOELSSON & BACKSTROM 1969)

The radiation do es in the common and external iliac veins during one course of treatment are given as mean values of the absorbed dose in pairs of LiF detectors and referred to the anatomy of the vessels Anatomic inequalities necessitate the values from right and left side being presented separately

The dose varied between 71 and 215 with a mean of 155 rad in the cranial part of the right common iliac vein with values of 138 to 691 mean 344 rad in the caudal portion of the same vein The lowest value was 246 and the highest 1 044 mean 470 rad in the cranial part of the external iliac vein while in the caudal part the variation lay between 292 and 1 031, mean 426 rad The dose in the cranial part of the left common iliac vein was between 78 and 273 with a mean of 158 rad The dose ranged between 204 and 491 mean 334 rad in the caudal part of the vein The dose varied between 302 and 699 mean 467 rad in the cranial part of the external iliac vein and in the caudal part of the external iliac vein the corresponding values were 312 to 664 with a mean of 445 rad The one detector located exactly beneath the inguinal ligament was always treated separately dose variation on the right side was 245 to 646 mean 321 rad and on the left side 242 to 465 mean 348 rad Detectors were sometimes placed in the inferior vena cava and doses averaging about 100 rad were registered One or two detectors were introduced in 6 patients into the middle part of the common iliac vein on the left side and in 4 patients into the right side the mean doses amounted to 260 to 280 rad Detectors were also dependent on anatomic variations put into the middle part of the external iliac vein i e 9

Table 3

Individual differences in dose rates between measurements in lithotomy and supine positions as percentages of values obtained with the patient in lithotomy position. Remote afterloading technique

	Difference in dose rate as percentage of lithotomy position value	
	Urinary bladder	Rectum
	+ 1	−21
	−27	0
	+22	−12
	+ 2	− 1
	−19	+10
	+ 9	+14
	+ 1	+72
	+ 2	− 4
	+ 4	+46
	−19	+75
	+ 7	+12
	+13	+94
	− 9	+ 6
	+ 2	−30
	0	− 3
	+19	− 1
	+ 9	+32
	+14	+33
Limits	−27 and +22	−30 and +94
Mean	+ 2	+18
S.E.	±3.1	±8.1

et coll. 1969). Primary radiation treatment consisted of two courses of intra-uterine (^{137}Cs, 600 mCi) and intravaginal (^{137}Cs, 600 mCi) applications, three weeks apart. The combined intravaginal and intra-uterine applicators were connected to the remote afterloading unit, Cervitron 7S (manufactured by Nuclesa). The dose rates at the posterior wall of the bladder and the anterior wall of the rectum, with the treatment sources in position, were measured with the Siemens Gammameter immediately after final fixation of the applicators to the patient. This was done with the patient supine, in which position the dose rates were also measured. This was considered necessary because in a separate study in 18 patients, treated with the applicator combination No. 1 considerable differences

occurred between the dose rates at the anterior wall of the rectum measured in lithotomy and supine positions (Table 3). The range of differences was large with a minimum of −30 % and a maximum of +94 % with a mean of +18 %. Six of the 18 patients exhibited differences of over +20 % and for 4 of them the difference amounted to more than +40 %. The mean difference of +18 % cannot be explained by inaccuracies in measurements, but suggests a true difference between dose rates in the two positions. No such differences were observed in dose rates on the posterior wall of the urinary bladder. For the detailed description of the procedure of dose rate measurement the reader is referred to a previous paper by JOELSSON & BÄCKSTRÖM (1969).

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Table 4

The radiation doses in rad (mean values and limits) during one course of intracavitary radiation treatment with the gold screened vaginal applicator of the afterloading unit together with an intra uterine stainless steel sound Bilateral catheters with thermoluminescence LiF detectors were employed in 8 patients

	Inferior vena cava	Common iliac vein			External iliac vein			Under inguinal ligament
		Cranial	Middle	Caudal	Cranial	Middle	Caudal	
Right side								
Mean	*93*	*155*	*282*	*344*	*470*	*526*	*426*	*321*
Limits	44—123	71—215	217—323	138—691	246—1 044	344—1 043	292—1 031	245—646
Left side								
Mean	98	158	263	334	467	507	445	348
Limits	54—134	78—273	165—339	204—491	302—699	357—676	312—664	242—465

detectors (6 patients) on the right and 10 detectors (8 patients) on the left side On the right side the doses were 344 to 1 043, mean 526 rad, and on the left side 357 to 676, mean 507 rad The dose contribution from diagnostic roentgen procedures amounted to 2 to 3 rad It should be emphasized that the accuracy of the thermoluminescence technique was about ± 8 % (standard deviation as percentage of the mean) A complete list of the dose determinations is given in Table 4

Discussion

A critical review of the factors influencing the dose distribution in intracavitary radiation treatment of carcinoma of the uterine cervix has been given by NEARY (1943, 1947) He concludes that little fundamental improvement in the distribution of the radiation dose can be expected from any variation of the basic Paris or Stockholm methods The broad features of dose distribution are similar in most existing techniques The only way to increase the dose in the regions of spread of disease near the pelvic wall is to increase greatly the irradiator activity in the uterus and the vagina This makes the protection of the rectum and bladder from overdosage of great importance NEARY used radium sources centrally in the vagina with platinum screens More recently FLEMING has introduced a similar central source vaginal applicator utilizing ^{60}Co radiation sources and heavy alloy metal (90 to 97 % tungsten) to shield the rectum (FLEMING & WIERNIK 1963) It appears that the lateral decline of doses is little inferior to that of the Manchester technique (BATES et coll 1968)

The vaginal applicator, which is used in connection with the remote after-

loading unit at Radiumhemmet, is ring shaped The radiation sources in the form of a source train can be dispersed mechanically to the lateral parts of the vagina ^{137}Cs with a gamma energy of 0 66 MeV is used and the activity of the sources is 600 mCi corresponding to 240 mg radium In the latest version of the vaginal applicator 18 carat gold screens are adapted to the anterior and posterior surfaces of the ring gold flanges are placed between the separate cesium sources and the sintered tungsten absorbers are maintained in the source train The dose distribution from the vaginal ring itself appears extremely satisfactory but when the intra uterine tube with its central sources is added to the system the dose distribution appears to be less satisfactory

The individual variation of absorbed doses is in general less marked with the afterloading technique than with the current Stockholm method The reasons are associated with the conformity in relation to the individual anatomy and the stability during treatment time achieved by attaching applicators to the patients with a bar and corset system The traction exercised by the flexible tubes connecting the intracavitary sounds with the storage container of the afterloading unit might be thought to cause changes in the position of the vaginal and uterine ends of the sounds no such changes were however observed The ball joint can be locked very firmly which prevents it from acting as a fulcrum on which the lever can turn In the current Stockholm technique the vaginal applicator is held in position by gauze packing in the vagina only and is not connected to the intra uterine applicator The fallacy of relying upon the gauze pack as an invariable spacer between the source and the rectum has been commented upon elsewhere (JOELSSON & BACKSTROM 1969)

A striking similarity in doses in the afterloading technique was evident on the right compared to the left side of the pelvis the highest mean value on the latter being less than 10 % higher than the corresponding value on the right side In the series of patients studied with thermoluminescence dosimetry during treatment according to the current Stockholm technique a 50 % difference in dosage from one side to the other was occasionally registered

Doses at the pelvic wall are often given in the literature relative to the dose in the middle of the paracervical triangle Careful determination of dose rates of clinical significance at the bladder base and anterior rectal wall is always performed at Radiumhemmet The dose values at these locations are therefore measured doses and are available for a comparison

The devices for securing the applicators in the afterloading technique interfere however with the introduction and movements of the probe of the Siemens Gammameter As the ball joint is located in the medial sagittal plane the Gammameter probe will invariably be directed either obliquely or be displaced laterally This obstacle causes difficulties in finding the small area of maximum

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dose rate The significance may be considerable as the dose rates are measured close to irradiators of high activity where the dose rate gradient is steep Only about 5 mm displacement of the cadmium sulphide crystal of the Gammameter sound can give rise to differences in dose rates of 30 to 40 %

It has been demonstrated earlier that the highest doses attained at the pelvic wall *during radium irradiation according to the current Stockholm method* were 22 % of the bladder dose and 20 % of the rectal dose (JOELSSON et coll 1969) The dose on the pelvic side wall in the present study was 18 % of the bladder dose and 26 % of the rectal dose This is a reflection of the fact that gold screens adapted to the vaginal applicator considerably reduce the dose rate at the anterior wall of the rectum At the base of the bladder, however, the contribution from the intra uterine radiation sources dominates because of the angulation of the uterine sound

Reference doses other than those in the bladder and rectum are not clinically available A comparison of the absolute figures of doses over one course of treat *ment is not to be recommended because of the differences in activities duration* of treatment and the use of absorbing metal screens In the current Stockholm technique the amount of radium in the uterine applicator varies between 53 and 74 mg, and in the vaginal applicator between 60 and 80 mg The irradiation time is between 25 and 28 hours for each course With the afterloading technique 600 mCi ^{13} Cs is applied both in the uterus and in the vagina and the treatment time is between 6 and 8 hours This means that the activity is increased by a factor of 3 6 and the treatment time is decreased by a factor of 3 8 If the data for the relationship between total dose and treatment time, published by LIVERSAGE (1969) on the basis of information given by MITCHELL (1960), McWHIRTER *(1936) and* COWELL *(1938) be taken into account, the treatment time should* have been still further reduced Such a reduction must be weighed against the reduced radiation dose to part of the tumor and surrounding tissue effected by *the gold screens When continuing the comparison* between the two series re ferred to above, it is interesting to find that the mean dose at the anterior wall of the rectum for the patients in the afterloading series was 20 % less than the corresponding dose over one course of treatment by the current Stockholm tech nique This figure is in accordance with LIVERSAGE s recommended reduction *in* the tumor lethal dose upon change of treatment time from 28 to 8 hours

Even if shielding devices inbuilt into the applicators can indeed be used as *means of reducing* the sharp decline in doses in lateral directions, this would mean that individualization of the treatment would be rendered considerably more difficult A great number of applicators of different sizes would have to be available

The use of heavier shields therefore appears to be of doubtful value The

employment of radiation sources with lower gamma energies e g [19] It would on the other hand change the situation The effective energy of 17 gamma ray lines is about 380 keV and the half value layer 2 2 mm in lead in comparison with 5 5 mm for ^{137}Cs These favourable data might outweigh the drawback of a rapid decay with a half life of 75 days

SUMMARY

Selective shielding with gold screens considerably increased the pelvic dose in relation to the rectal dose in the treatment of carcinoma of the cervix uteri with two different intra cavitary afterloading applicator systems The vaginal part of the present applicator device represents one phase in continued efforts towards optimum dose distribution Different types and sizes must become available so as to facilitate individualized treatment by the remote afterloading technique

ZUSAMMENFASSUNG

Es konnte gezeigt werden dass die selektive Abschirmung mit Goldschirmen die Tiefen dose im Becken wesentlich verbessert im Vergleich mit der Dose die das Rektum erhält wenn man das Cervixcarcinom des Uterus mit zwei verschiedenen Hinterladermodellen von Applikatoren bestrahlt Der verbesserte in der Scheide liegende Anteil des Applikators bedeutet ein Fortschritt in der besseren Dosenverteilung im Becken. Verschiedene Grossen und modifizierte Modelle mussen geschaffen werden um in jedem individuellen Fall die Hinterladungstechnik mittels Fernkontrolle durchzufuhren

RÉSUMÉ

La protection selective par des écrans en or augmente considerablement la dose pelvienne *par rapport a la dose rectale* dans le traitement du cancer du col de l uterus par deux systemes différents d applicateurs intracavitaires charges *apres leur mise en* place La partie vaginale de cet applicateur represente un des elements d une serie continuee de perfectionnements en vue d une distribution de dose optimale *Il faut creer differents* types et differentes tailles d applicateurs pour faciliter l'adaptation a chaque malade du traitement par applicateurs charges à distance *apres leur mise en* place

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FROM THE RADIOTHERAPY CLINIC (PROF L. R HOLSTI), UNIVERSITY CENTRAL HOSPITAL HELSINKI AND THE SEMICONDUCTOR LABORATORY (PROF T STUBB) STATE INSTITUTE FOR TECHNICAL RESEARCH OTNAS FINLAND

NEUTRON RADIATION PRODUCED BY THE 32 MeV ROENTGEN BEAM OF A MEDICAL BETATRON

by

KAJ ERIK LOFGREN and ERIK SPRING

Nowadays medical betatrons are used mainly for the production of high energy roentgen rays and most radiotherapeutic treatment is given with the maximum energy of the machine

Neutron radiation is created when the energy of the roentgen rays exceeds the threshold energy for a (γ n) reaction this is unwanted radiation when patients are treated with either roentgen rays or electrons from a betatron The most important reactions concerned in the irradiation of human tissue are those in ^{12}C ^{14}N and ^{16}O with the threshold energies 18 72 10 55 and 15 67 MeV respectively When the betatron beam strikes heavy materials of the collimating systems or other parts of the machine neutron radiation is also produced

Difficulties have been encountered in earlier investigations of the neutron radiation in view of the strong electron or roentgen radiation that affects the measurements These difficulties can be overcome by application of a detection method that is not disturbed by other radiations The solid state track detectors used in this study are suitable in this respect

The purpose of this investigation was to develop a solid state track detector,

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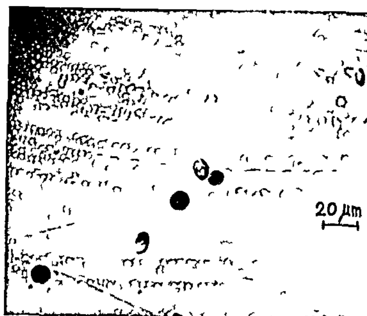


Fig 1 Etched tracks in soda lime silica glass

intended especially for measurement of the neutron contamination in the beam of the medical betatron and in the neighbourhood of the machine Furthermore, a great deal of care was given to the determination of the neutron radiation to the patient under treatment

Solid state track detectors When heavy particles such as fission fragments or atomic nuclei, traverse certain insulating solids they leave trails of radiation damage, which become evident as tracks when examined under a transmission electron microscope (FLEISCHER et coll 1965) The trails of radiation damage are chemically attacked much more rapidly in an etching solution than is the undamaged part of the solid (PRICE & WALKER 1962) After the etching procedure, the tracks are visible under an optical microscope they appear black in normal bright field illumination and white when viewed on a dark field

The solid state detector also provides a possibility of detecting neutrons by allowing them to traverse a neutron fissionable material which is put close to a track detecting solid in which the resultant fission fragments cause damage

The solid state track detector is usable as a radiation dosimeter, as the track intensity, that is, the number of tracks per unit area is a measure of the radiation *Consequently* the detector can be calibrated by the aid of known neutron sources

After experiments with a number of different materials (e g biotite mus o-vite polycarbonate resin) glass was found to be the best material for these measurements The detector material con i ted of an ordinary soda lime silica glass in which uranium oxide had been mixed to the amount of 1 % uranium The detectors were prepared at the Nuutajarvi Notsjo Glass Wärtsila Corpora tion The detectors measured 17 mm × 30 mm × 2 mm

The etching was effected in 38 to 40 % hydrofluoric acid for 12 seconds The temperature of the etching solution was 65° C A criterion in the deter mination of the etching parameters was that the tracks should be clearly visible under an optical microscope with a magnification of × 900 In counting the tracks only those with a sharp base were accepted An example of the tracks is reproduced in Fig 1

Detection method The following formula is valid for the number of fissions K

$$K = \sigma_f N x \Phi \tag{1}$$

where σ_f is the fission cro s section N *is the number of fissionable atoms within* depth x of the detector material x *is the mean path of a fission fragment for* producing a track visible after etching *and* Φ *is the total neutron fluence*

The track density T i e the number of tracks per cm², is given by the formula

$$T = \beta K = \beta N x \sigma_f \Phi = F \sigma_f \Phi \tag{2}$$

where β is the efficiency of the detector and F ($= \beta N x$) *in this* paper is called the detector constant (which is mainly dependent upon the etching parameters reagent time *temperature* and type of glass) It has been found that the efficiency of gla s detectors is about 0 40 (DEBEAUVAIS et coll 1964)

The detector con tant can be determined experimentally by irradiation of the detectors by monoenergetic neutrons of known energy By determination of the track density the F value is obtained from formula (2) When the neutron energy varies the effective cross-section $\bar{\sigma}_f$ and the integrated fluence Φ have to be determined from the energy distribution of the neutrons

The detectors were calibrated against two Am Be sources (Department of Physics University of Helsinki and Institute of Radiation Physics Helsinki) With the neutron source placed in water, the neutrons are in thermodynamical equilibrium with the water when the distance is greater than 2 cm and it can be assumed that the spectrum consists of a Maxwell distribution and a tail, which is $1/E$-distributed where E is the neutron energy (BECKURTS & WIRTZ 1964) A temperature of 293° K corresponding to a neutron energy of 0 025 eV was used in calculation of the Maxwell distribution The cross-sections for

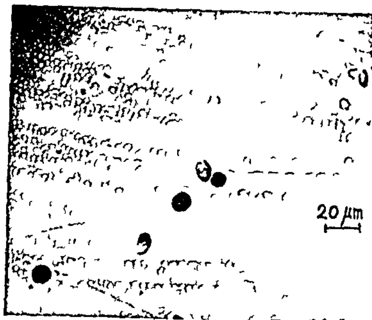


Fig. 1. Etched tracks in soda lime silica glass.

intended especially for measurement of the neutron contamination in the beam of the medical betatron and in the neighbourhood of the machine. Furthermore, a great deal of care was given to the determination of the neutron radiation to the patient under treatment.

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Table 1

Results of measurements in a water phantom

Depth (cm)	$\bar{\sigma}_{fm}$ (mb)	$\bar{R}_c$ (rem/n/cm)	$\overline{QF}$ (rem/rad)	Neutron dose equivalent (mrem/rad roentgen dose*)		Neutron dose (mrad/roentgen dose*)	
				Inside beam	Outside beam	Inside beam	Outside beam
0 3	116	8 06 10 *	7 6	0 071 ±0 005	0 0 1 ±0 006	0 0028 ±0 0007	0 0028 ±0 0008
5 5	52 5	3 58 10	5 0	0 029 ±0 007	0 024 ±0 006	0 0058 ±0 000?	0 0048 ±0 0002
13 5	55 9	3 35 10	4 8	0 023 ±0 005	0 019 ±0 005	0 0048 ±0 0001	0 0040 ±0 0001
20 5	55 9	3 35 10	4 8	0 072 ±0 005	0 027 +0 004	0 0046 ±0 0001	0 0056 ±0 0001

*Roentgen dose measured in water at the maximum point of the depth dose curve

$\nu = (6\,9 \pm 0\,2)\ 10^{17}\ a^{1}$ (FLEISCHER & PRICE 1964) This implies about 25 fissions per hour in one gram of ^{235}U

The final formula for the neutron fluence is

$$\Phi_m = k\ \ T\ \ - T_f \tag{4}$$

where T_f corresponds to the track density caused by spontaneous fissions

If photon energies that exceed 5 MeV are used photofission reactions are also possible although the cross sections for these reactions are so low that the reactions do not disturb the measurements An estimate indicates that only about 3 per thousand of the tracks originate from these reactions

Finally when the neutron fluence is determined the corresponding dose equivalent DE (rem) can be calculated

$$DE = \bar{R}_c\ \ \Phi_m \tag{5}$$

and the corresponding dose D (rad)

$$D = \frac{DE}{\overline{QF}} = \frac{\bar{R}_c \Phi_m}{\overline{QF}} \tag{6}$$

where effective values of the conversion factor R_c (rem/(neutrons/cm)) and the quality factor QF must be used in view of variations in the neutron energy The $\bar{R}$ calculations were effected with the neutron energy spectra determined and the R_c-values based upon information given in NBS Handbook No 63 (1957) The effective quality factor was calculated with the aid of values given in this handbook as well as in ICRP No 4 (1964)

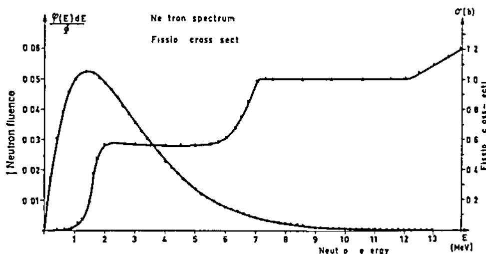


Fig 2 Spectrum of the neutron radiation from the betatron and cross sections for natural uranium calculated with the aid of the values published by SCHMIDT (1962)

natural uranium (Fig 2) have been calculated with the aid of the values published by SCHMIDT (1962) and HALPERN (1959)

When the integrated neutron fluence Φ and the effective cross-section $\bar{\sigma}_f$ are known, the detector constant F is determinable by the application of formula (2) The numerical integrations were made in accordance with the Weddles rule (MARGENAU & MURPHY 1964), and $F = (0\ 66 \pm 0\ 10)$ tracks/(neutrons barn) was obtained

It is now possible to determine the total neutron fluence Φ_m measured with the detectors concerned The following formula is valid

$$\Phi_m = \frac{T_m}{\bar{\sigma}_{fm}\ F} = k\ T_m \qquad (3)$$

where $\bar{\sigma}_{fm}$ is the effective cross section for the neutron fluence under investigation, F the detector constant obtained above, T_m the measured track density, and $k = 1/\bar{\sigma}_{fm}\ F$

The effective cross-section $\bar{\sigma}_{fm}$ must be determined separately for every measurement if the measuring conditions are changed The calculations in this study were based upon values reported by SCHMIDT (1962), and the cross sections obtained are given in Table 1

In the application of formula (3) a correction for spontaneous fission in ^{238}U must be taken into consideration, as the spontaneous fission decay constant

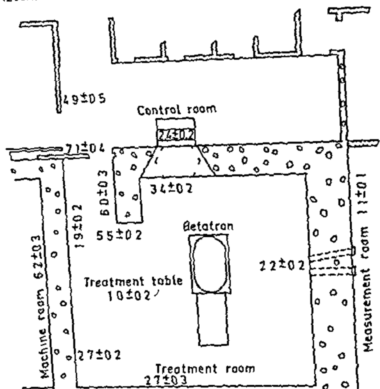


Fig 4 Schematic figure illustrating the neutron dose equivalents in mrem/h measured at different points in the surroundings of the betatron Asterisk indicates measurement under the treatment table

the same value was obtained both inside and outside the beam The measurements were made up to a distance of 7 cm outside the field used The values were higher at the central ray i e 0 039 ± 0 006 mrem/R and (5 1 ± 0 7)10³ (n cm)/R respectively

Measurements in a water phantom The neutron dose in water — tissue equivalent material — consists of the neutrons measured earlier together with the neutrons originating from $(\gamma\, n)$ reactions in tissue, mostly from the $^{16}O(\gamma\, n)^{15}O^*$ reactions

The neutron spectrum used for the calculation of $\overline{\sigma}_{fm}$ $\overline{R}_c$ and $\overline{QF}$ was a sum of a Maxwell distribution and a neutron spectrum of the $^{16}O(\gamma\, n)^{15}O^*$ reaction presented by Fuchs et coll (1965) The neutron spectrum varies for different depths in the water (Beckurts & Wirtz 1964) When the depth exceeds 10 cm it can be assumed constant

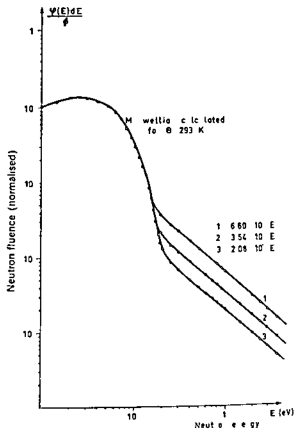


Fig 3 Calculated neutron spectra in water at (1) 5.5 cm (2) 13.5 cm and 20.5 cm depths and (3) at a distance of 7.0 cm from an Am Be source

Measurements

All the measurements were made with SSD = 80 cm, field 10 cm × 10 cm and a roentgen energy of 32 MeV In every single measurement, the roentgen dose corresponded to a dose of 860 rad measured in water at the maximum point of the depth dose curve

Measurements in air The neutrons originate from (γ,n) reactions in the betatron collimator and other heavy material of the machine The energy spectrum must be calculated theoretically (Blatt & Weisskopf 1952) It is assumed that the spectrum is Maxwell distributed with a temperature of $\Theta = 1.4$ MeV (± 7 %), where the errors shown are the possible maximum deviations, by reason of the approximations made in the calculations (Fig 2) The effective cross section $\bar{\sigma} = 86.9$ mb, $\overline{R}_c = 7.66 \cdot 10^{-9}$ rem/(n cm^2), and $\overline{QF} = 7.4$ were determined

An average value of 0.014 ± 0.006 mrem/R was obtained with the exposure measured at the central ray 80 cm from the anticathode This corresponds to a neutron fluence of $(1.8 \pm 0.7) 10^3$ (n/cm^2)/R It should be observed that

measurement are indicated in the figure Most of the results are of the order of the allowed maximum dose equivalent 2 5 mrem/h, which of course also includes all other types of irradiation No one is allowed to stay in the neigh bourhood of the door of the treatment room or in the machine room during the irradiation

Discussion

The results obtained in this study are compared in Table 2 with those published earlier The differences in the results are difficult to explain Of course all the other measurements are based upon activation analysis and at least some of them are influenced by the strong roentgen beta or proton radia tion present during the measurements The solid state track detector should not be affected by radiation other than neutron

The results presented here have moreover been checked with a BF_3 proportional counter (ANDERSSON & BRAUN 1964) developed by AB Atomenergi and made by 20th Century Electronics With this counter 115 ± 5 mrem/h was obtained in air at the central ray 80 cm from the anticathode The cor responding value deduced from the measurements with the detectors employed was 98 ± 16 mrem/h Measurements made behind 8 2 cm water gave 80 ± 10 mrem/h (proportional counter) and 75 ± 18 mrem/h (SST detectors) The SSTD measurements are also in agreement with scintillator counter (boron enriched scintillator) measurements made by RYTILA (1962)

The dose equivalent to the patient caused by the neutron radiation is 0 11 0 16 rem during a treatment of 6 000 rad This is a small value (0 0037 0 0053 rad $QF = 30$) even for the eyes which are sensitive to neutron radiation

Acknowledgement

The authors wish to thank the Wartsila Corporation Nuutajarvi Notsjo Glass for preparing the detectors used in this study

SUMMARY

Solid state track detectors consisting of 1 % uranium soda lime silica glass have been used for investigation of the neutron fluence and the corresponding neutron doses and dose equivalents inside outside and in the neighbourhood of the roentgen beam of the medical betatron

ZUSAMMENFASSUNG

Kernspurendetektoren aus festem Material bestehend aus 1% igen Uran Soda Kalk Kiesel Glas wurden fur Untersuchung n der Flussdichte von Neutronen und der entspre chenden Neutronendosen und der Dosis Aquivalente innerhalb ausserhalb und in der Nahe des Strahlenganges eines medizinischen Betratrons verwendet

Table 2

Measurements of neutron radiation in the roentgen ray beam of a betatron

Investigation	Energy (MeV)	Neutron dose equivalent per roentgen exposure (mrem/R)	Neutron fluence ((n/cm²)/R)	Betatron
WAFFLER	31	2.8	1.3 · 10⁴ at 1 m	BBC-31 Medical
LAUGHLIN (1951)	23	3.0	4.2 · 10⁴ at 0.84 m	University of Illinois Medical
POHLIT (1960)	31	2.4	3.1 · 10⁵	BBC 31 research
FROST & MICHEL (1964)	34	15	1.7 · 10⁵ at 1 m	Asklepitron
BRENNER (1965)	32	1.0 – 1.3*	(1.3 – 1.7)10⁵ at 1 m	Asklepitron
Present	32	0.039	5.1 · 10³ at 0.8 m	Asklepitron

Asterisk denotes: calculated with an effective value of $R_c = 7.66 \cdot 10^{-9}$ rem/(n/cm²)

The spectrum at a depth of 0.3 cm is assumed to be a Maxwell distribution, with $\Theta = 1.4$ MeV and an added tail of photoneutrons. For depths of 5.5, 13.5 and 20.5 cm, the distribution consists of a Maxwell distribution ($\Theta = 0.025$ eV = 293° K), and an $1/E$ tail. These spectra are shown in Fig. 3, together with the spectrum used for the Am-Be source in water. The influence of neutrons from ^{16}O (n) $^{15}O^*$ reactions is accounted for in the $1/E$ tail, since these neutrons have not reached thermodynamical equilibrium with the water.

All the values obtained for $\bar{\sigma}_{t,n}$, $\bar{R}_c$, $\overline{QF}$ and the measurements made with the solid state detectors, are contained in Table 1. The neutron doses and dose equivalents correspond to a roentgen dose of 1 rad at the maximum point of the depth dose curve, i.e. at a depth of 5 cm in water.

Table 1 illustrates that the neutron radiation is inclined to be similar within and outside the beam. It can be said that on the average the neutron dose equivalent is 0.0045 ± 0.0004 mrem per 1 rad roentgen dose at the dose maximum. It must be emphasized that the measurements are made only to a depth of 20.5 cm in water, and to a distance of 7 cm outside the beam, i.e. the irradiation field.

Measurements during treatment. Detectors were placed at different points in and outside the treatment room. A schematic figure of the room, the betatron, and the surroundings is reproduced in Fig. 4. The detectors were kept there for three weeks, with 40 hours treatment time per week. The results of

FROM THE DEPARTMENT OF TUMOR BIOLOGY, THE MEDICAL SCHOOL OF KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

SURVIVAL CHARACTERISTICS OF MAMMALIAN CELL LINES AFTER SINGLE OR MULTIPLE EXPOSURES TO ROENTGEN RADIATION UNDER OXIC OR ANOXIC CONDITIONS

by

BO LITTBRAND

Much information has accumulated during the past decade on the radiation survival of a great variety of mammalian cell lines with the clonogenic ability of the cell as an index (ELKIND & WHITMORE 1967). Most of the observations concern the survival characteristics after irradiation under aerobic conditions. Few investigations have treated the problem of the dose effect relationship with irradiation in hypoxia or in a total absence of oxygen. The data in the relatively few publications have often been inconsistent. Attempts to resolve the contradictions (e.g. at the 1st Gray Memorial Meeting in London 1967) have met with only partial success. The differences in the biologic materials combined with the differences in the experimental procedures, especially those that concern control of hypoxic conditions, present serious obstacles to a conjoint interpretation of the available data.

The investigations to be described were conducted with the aim of comparing the reactions of different cell lines to oxic and anoxic roentgen radiation under

Submitted for publication 29 May 1969.

RÉSUMÉ

Des détecteurs à l'état solide faits de verre de silicate de soude et de chaux à 1 % d'uranium ont été utilisés pour étudier la fluence des neutrons et les doses correspondantes des neutrons ainsi que les doses équivalentes à l'intérieur, à l'extérieur et dans le voisinage du faisceau d'un bétatron médical.

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the strain was discontinued New cultures were established from earlier samples which were preserved frozen in a tumor bank

Irradiation Radiation was generated by a Siemens roentgen unit at 190 kV and 15 mA the radiation was filtered by 1 5 mm Al inherent filtration and a 1 0 mm Al additional filter, half value layer 0 95 mm Cu The dose rate was 235 R/minute at the bottom of the culture dishes which were always placed at a distance of 43 cm from focus The dose delivered was measured by a Philips integrating dosimeter Pyrex petri dishes were used in all the tests The correction factor for back scatter established from survival measurements of Chinese hamster cells while irradiated on glass versus plastic was 1 35 ± 0 05

Experimental procedure Suspensions of monodispersed cells were prepared from 6 to 8 days old cultures growing in a non confluent monolayer on the surface of stoppered milk dilution bottles The cells were readily dissociated by treatment with a 0 5 % trypsin solution at a temperature of 37 °C for 10 minutes After establishing the cell concentration in a haemocytometer, an appropriate number of cells was explanted in pyrex dishes of 6 cm diameter containing 4 to 6 ml of nutrient medium The number of cells was chosen so that about 100 cells were expected to survive exposure to a particular roentgen dose The dishes were incubated for 3 to 4 hours at 37 °C in a moist atmosphere with 5 % carbon dioxide in air to maintain a pH of 7 0 to 7 2 After incubation, the cells were irradiated and reincubated for 10 days with two medium changes the first taking place 2 to 3 and the second 6 to 7 days after irradiation The clones that developed were fixed and stained in situ Those that comprised more than 50 cells were counted and taken to represent the surviving fraction of the explanted population Mean values were routinely calculated from three (irradiated cultures) or six (unirradiated controls) replicate dishes The fraction of cells surviving irradiation was expressed as the percentage of the unirradiated controls in the same experiment The plating efficiency of unirradiated controls was defined as the percentage of 100 to 200 cells that grew into macroscopic colonies in 10 days

Roentgen irradiation was administered at a temperature of about 27 C in an air tight plastic chamber of 2 liter volume The chamber had two orifices of 6 mm diameter for the in and outlet of gases The lids of the culture dishes were removed and the nutrient medium withdrawn to such an extent that no more than 1 ml fluid to cover the cells was left The chamber was filled with either oxygen or argon both supplemented with 1 25 % carbon dioxide the flow rate being adjusted to 6 liter/minute The gases passed through pre equilibrated water warmed to a temperature of 38 C Argon with an oxygen contamination less than 2 ppm was used and led to the chamber through a system of stainless

identical experimental conditions In view of recent observations (CHAPMAN et coll 1968, LITTBRAND & REVESZ 1969) particular attention was paid to the control of the oxygen concentration at a level previously thought to be of minor importance in radiobiologic experiments The design of the experiments received special attention to ensure that the data should be suitable for statistical analysis A closer knowledge of the cellular reactions to hypoxic or anoxic radiations may be both of theoretical and practical interest It may help to throw further light on the mechanism of the biologic actions of radiations and to develop more effective plans for the treatment of neoplasms by more effectively controlling their portions with a deficient oxygen supply

Materials and Methods

Cell cultures Cultures of Chinese hamster cells (V 79 379 A), an Ehrlich ascites tumor (LLD), and three substrains of the latter, denoted ELT, SELD clone a and SLI D clone g, were used The isolation of the clones and their characteristics have already been desribed (HAUSCHKA et coll 1957, REVESZ, GLAS & HILDING 1963b) The ELT substrain has a duplicated, i e hypertetraploid, chromosome set in relation to ELD and is also characterized by an increased radioresistance under certain in vivo conditions, as compared to the original cell strain (GLAS & REVESZ 1963) The cells in clone a and clone g have chromosome sets in the same poidy region as ELD but differ in their sensitivity to radiation in vivo, clone a being as sensitive as LLD and clone g less sensitive (REVESZ et coll 1963b)

The Chinese hamster cells were received from Dr Elkind of Bethesda in 1964 and were already adapted to growth in culture The cultures of ELD and its substrains were established from ascites material grown in vivo Ascites of a particular strain freshly withdrawn was dispensed in amounts of 0 1 ml in stoppered Pyrex milk dilution bottles of 120 ml volume The culture medium consisted of Eagle s medium in Earle s solution (EAGLE 1959) supplemented with 15 % fetal calf serum and antibiotics After a period varying from 7 to 18 days, multiple foci of vigorously growing colonies became visible, firmly attached to the bottom of the culture flasks The colonies were detached and dispersed by gentle trypsin treatment the cells being cultivated in serial passages by transfer of 10^4 cells at weekly intervals and by medium changes every second or third day In order to protect against development of new substrains, prone to occur in prolonged serial cultures in vitro (CAILLEAU & COSTA 1961 NIELSEN 1967), the chromosomal constitution of the cell population was consequently examined at intervals of about ten successive passages as described previously (REVESZ et coll 1963b) and whenever changes were noted the propagation of

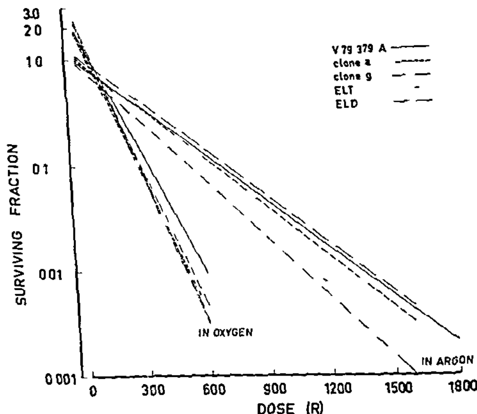


Fig 2 Survival regression of the cell lines after exposure to roentgen radiation in argon or oxygen The numerical value of the regression parameters and their S E is presented in Table 1

larity between the plating efficiency of the cells that were exposed in either argon or oxygen indicates that the difference in the gas phase does not affect the survival of unirradiated cells to any appreciable extent under the conditions used (cf Tables 3 4 and 6)

Statistical considerations A main consideration in the design of the experiments concerned the suitability of the data expected for a statistical evaluation of their significance In determining the variance of the slope constant ($1/D_0$) and the extrapolation number (n) the statistical requirement for independent observations is satisfied if several repeat tests of the survival regression are performed then the $1/D_0$ and n of each survival curve may be regarded as a single observation The statistical degrees of freedom are determined solely by the number of

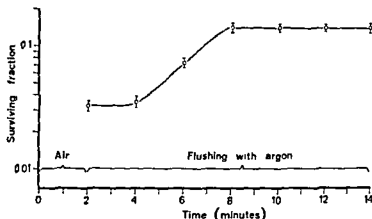


Fig 1 Survival of Chinese hamster cells exposed to 500 R during different periods in the course of a 12 min period of flushing of the irradiation chamber with argon The results are from 6 repeat tests The points with S E stand at the end of the 2 min irradiation period The gas flow was 6 liter/minute and the chamber had a 2 liter volume

steel and glass tubes with joints sealed impermeably to oxygen from the atmosphere The gas outflowing from the radiation chamber passed a water lock and was routinely monitored by an Elcoflux oxygen analyser (Dr Thiedig & Co KG, Berlin)

The flushing period of the chamber was 12 minutes in the early tests, and 14 minutes in the final tests The irradiation period varied with different doses and was adjusted to terminate at the end of the flushing period and never started before 6 minutes of flushing

The survival of Chinese hamster cells treated with 500 R at different times in the course of a 12 min flushing of the irradiation chamber with argon is illustrated in Fig 1 For flushing times of 4 minutes or longer the survival was always similar, indicating that equilibration between the gas phase and the liquid phase that surrounded the cells was complete within 4 minutes This finding is in agreement with theoretical calculations discussed elsewhere (LITTBRAND & REVESZ 1969)

After radiation exposure the dishes were removed from the chamber, refilled with 5 ml fresh medium and incubated in air and carbon dioxide The exposure procedure with multiple irradiations was repeated after various time intervals Unirradiated control cultures were sham irradiated under the same conditions as the others The cultures which received a single exposure dose and belonged to the same experimental series in which the effect of the same dose split into fractions was studied were also sham irradiated at appropriate intervals The simi

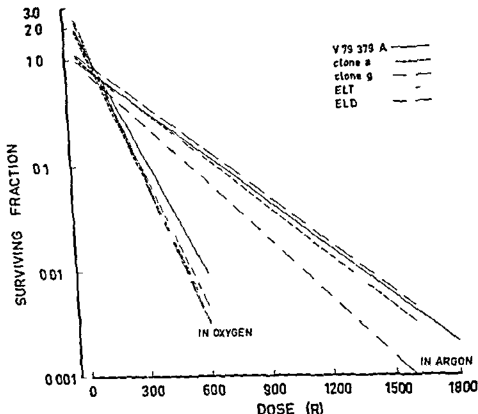


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Table 1

Regression parameters of the radiation survival of different cell lines as established by least square analysis of data in repeated tests — Two to four different roentgen doses were used to establish the survival in each test

Cell line	Gas phase during radiation exposure	Plating efficiency Mean ± S.E. (%)	Number repeat tests	Total number surviving fractions determined*	Slope constant of the survival regression Mean ± S.E. (^{e}log units/100 R)	D_0 (R)**	Extrapolation number*** Mean ± S.E. (^{e}log units)	Arithmetic value of mean
I I D	Argon	76±5	17	36	−0.429±0.014	233	−0.020±0.143	0.98
	Oxygen	83±6	7	20	−1.062±0.031	94	0.692±0.158	2.00
FI F	Argon	35±2	18	52	−0.362±0.009	276	0.021±0.080	1.02
	Oxygen	41±6	7	23	−1.025±0.032	98	0.780±0.174	2.18
SELD clone a	Argon	41±3	20	54	−0.427±0.011	234	0.227±0.170	1.26
	Oxygen	41±2	16	48	−1.048±0.033	95	0.740±0.078	2.10
SFI D clone g	Argon	48±4	24	68	−0.340±0.016	294	0.030±0.225	1.03
	Oxygen	31±1	13	38	−1.087±0.026	92	0.833±0.110	2.30
Chinese hamster cells	Argon	76±4	16	59	−0.354±0.009	282	0.037±0.083	1.04
	Oxygen	64±9	6	18	−0.885±0.020	113	0.632±0.076	1.88

*The surviving fraction was determined from the mean value in three irradiated and six control replicate cultures

**Reciprocal of the slope constant indicating the increment of radiation dose which reduces the surviving fraction to 37%

***The intersection of the statistical regression line with the ordinate at zero dose

separate survival curves regardless of the number of survival determinations which build up any of the curves The survival determinations pertaining to one individual survival curve are dependent upon a common control, the same particular conditions regarding the quality of the medium, pH and temperature variations which may bias all the observations within a test and do not satisfy, therefore, the requirement for independent data

These considerations led us to perform a series of at least five repeat tests for determining the parameters of the survival regression of a particular cell line after irradiation In each test, the survival is in general established after exposure of cells in replicate dishes to two or three different roentgen doses These are of such a magnitude that the dose effect relationship can be assumed

Table 2

The ratio and its standard error between the slope constant of the oxic and anoxic survival regressions of the five cell lines tested — Data presented in table 1 were used for calculations — S E was approximated by formula (1) given in the text

Cell line	Ratio between the slope constants of the oxic and anoxic survival regressions Mean ± S E
ELD and sub strains	
ELD	2 48 ± 0 11
ELT	2 83 ± 0 12
SELD clone a	2 45 ± 0 07
SELD clone g	3 20 ± 0 12
Chinese hamster cells	2 50 ± 0 09

to be exponential The survival data even after such a few exposure doses are obviously sufficient for the statistical determination of a linear survival regression by least square analysis Since the confidence limit of the regression line is inversely related to the difference between the exposure doses (PORTER 1963) one of the doses is chosen, as a routine closest to a possible shoulder region of the expected survival curve, and another dose to give the minimum survival (in the range 10^{-3}) still detectable by our method with sufficient accuracy A third dose with a magnitude between the two extremes is also given to permit the control of the linearity of the survival regression The series of slope constants and extrapolation numbers obtained in repeat tests are then used for the calculation of the mean and the variance of these survival parameters and for the evaluation of their statistical significance

Results

Single exposures In a series of repeat tests cultures of Chinese hamster cells, ELD and its derivatives ELT SELD clone a and clone g were exposed to radiation in the dose range 200—600 R in oxygen or 800—1 800 R in argon

Assuming an exponential decrease of the survival in the dose ranges used (ELKIND & WHITMORE 1967) the survival regression was determined by least square analysis in each test and the mean regression of the different test groups calculated The results are illustrated in Fig 2 The numerical value of the regression parameters are summarized in Table 1

The reciprocal of the slope constant of the survival regressions (D_0) indicating the dose that reduces survival to 37 per cent gave values between 92 to 98 R

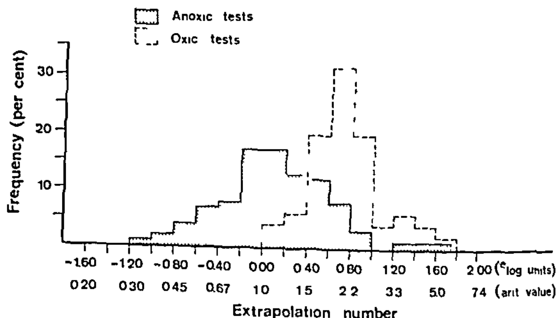


Fig. 3. Frequency distribution of the extrapolation number of the survival regressions calculated for each of the 49 oxic and 95 anoxic tests presented in Table 1.

when irradiation of ELD and its substrains was made in oxygen, and between 233 to 294 R when these cell strains were exposed to radiation in argon. The differences between the slope constants of the oxic cells were, as found by t test analysis, not significant. When treated in argon the slope constant of ELD and clone a differed significantly from ELT and clone g ($p < 0.001$), but the difference between ELD and clone a or ELT and clone g was not significant. The D_0 for Chinese hamster cells was 113 R in oxygen and 282 R in argon.

The intersection of the regression line of the individual tests with the ordinate at zero dose gave extrapolation numbers the log frequency distribution of which did not deviate from a normal distribution (cf. Fig. 3). The means were in the region of 2 after oxic treatments and close to unity after the anoxic treatments. When tests performed with the same cell strain are considered the difference between the mean extrapolation number in oxygen and in argon is significant ($p < 0.001$ or < 0.02).

The ratios between the slope constants of the survival regressions after oxic and anoxic irradiation are presented in Table 2, the data given in Table 1 being used for the calculation. The S.E. of the ratios were approximated by the following formula

$$S_P = \sqrt{\frac{R^2_0}{R^2_A}\left(\frac{S^2_0}{R^2_0}+\frac{S^2_A}{R^2_A}\right)\left(1+\frac{9S^2_A}{R^2_A}\right)} \qquad (1)$$

Table 3

Survival of one hundred Chinese hamster cells after exposure to roentgen doses in the range 50—150 R in either the presence or absence of oxygen — The mean plating efficiency was 96 ±3 and 95±2 per cent in the tests with argon and oxygen respectively

Number of repeat tests		10				19					
Exposure dose (R)		50		75		100		125		150	
Gas phase during radiation exposure		Ar	0	Ar	0	Ar	O_2	Ar	O_2	Ar	0
Surviving fraction ( )	Arithmetic value of mean	93.8	92.6	90.6	88.4	87.0	83.6	80.3	80.8	79.1	73.1
	Mean ± S E (log units)	4.541 ± 0.025	4.529 ± 0.033	4.506 ± 0.033	4.482 ± 0.023	4.466 ± 0.015	4.426 ± 0.021	4.386 ± 0.028	4.392 ± 0.022	4.371 ± 0.019	4.293 ± 0.021
Ratio between surviving fractions in argon and oxygen Mean ± S E (*log units)		0.012 ± 0.041		0.024* ± 0.042		0.040 ± 0.023		—0.007* ± 0.031		0.078** ± 0.024	

* P (probability of no difference from zero) > 0.05
** P<0.01

where S_R is the S E of the ratio between the respective slope constants, R_0 and R_A are the means of the slope constant of the survival regression obtained in repeat tests in oxygen and argon and S_0 and S_A are the S E of R_0 and R_A respectively

In comparing the ratio between the oxic and anoxic slope constants of ELD with the ratios of its sub strains the t test analysis will indicate that ELD or clone a has a ratio with a significantly smaller value than ELT or clone g ($p < 0.001$ or < 0.05) The value for ELT differs significantly from that of clone-g ($p < 0.05$) but the difference between the ratio of ELD and clone a is not significant

Effect of doses below 150 R The difference between the extrapolation numbers after oxic and anoxic irradiation implies a crossing of the regression lines As can be seen from Fig 2 the crossing occurs in a dose range around 120 R for each cell strain In this dose range therefore oxygen may not enhance the

Table 4

Survival of Chinese hamster cells after exposure in argon or oxygen to either a single dose of 300 R or to the same dose divided into three equal fractions and separated by a time interval of three hours — During the intervals the cells were incubated in air and carbon dioxide — The data are from 13 repeat tests

Exposure dose (R)	Treatment in oxygen			
	Cells exposed	Plating efficiency Mean ± S E (%)	Surviving fraction	
			Mean ± S E (-log units)	Arithmetic value of mean
3 × 100	300		3.5[illegible]9 ± 0.041	34.1
		98 ± 3	[illegible]	
300	300		2.9[illegible]5 ± 0.048	19.[illegible]

radiosensitivity of the cells After larger doses on the other hand the oxygen enhancement ratio defined by the ratio between the dose in oxygen and in anoxia which produces identical survival will obviously increase (cf Fig 2) It can be calculated (REVESZ & LITTBRAND 1967a) that the maximum oxygen enhancement ratio will be defined by the ratio between the slope constant of the oxic and anoxic survival regressions (cf Table 2)

The effect of oxygen was examined in two series of experiments on the survival around and below the dose range where the oxic and anoxic regression lines cross Chinese hamster cells in one series were exposed to a single radiation dose in the range 50 to 150 R in the presence or absence of oxygen The results are summarized in Table 3 The presence of oxygen does not seem to exert any major influence on the surviving fraction after exposure to a dose of 125 R or less The differences between the oxic and the anoxic groups are statistically insignificant On the other hand, the surviving fraction is significantly smaller ($p < 0.01$) after exposure to 150 R in oxygen than after treatment with the same dose in argon

The failure of oxygen to enhance cellular radiosensitivity when treatment is given with relatively small radiation doses was put to a further and more rigorous test in another series of experiments Chinese hamster cells were exposed three times to a dose of 100 R in the presence or absence of oxygen with an interval of 3 hours between the exposures Cultures treated under identical conditions with a single dose of 300 R and two subsequent sham irradiations were used as particular controls The results are presented in Table 4 The surviving fractions were of about the same magnitude after triple exposures when either oxygen or

Table 4 *(cont.)*

Treatment in argon				Ratio between survival fractions in argon and oxygen	
Cells exposed	Plating efficiency Mean ± S E ( )	Surviving fraction		Mean ± S E (elog units)	Arithmetic value of mean
		Mean ± S E (elog units)	Arithmetic value of mean		
300		3 568 ± 0 036	35 5	0 039 ± 0 040	1 04
	9[illegible] ± 4			1 4	
300		3 536 ± 0 040	34 3	0 581 ± 0 043	1 79

argon was the gas phase. On the other hand, when a single treatment was given with the same total dose, the survival was larger by a factor of 1 79 in argon than in oxygen. The survival after treatment with triple doses may also be calculated as 1 78 times larger than after treatment with a single dose in the presence of oxygen. When oxygen was absent, only an insignificant difference was noted in the effect between a single and triple radiation treatment.

Estimations of the oxygen enhancement ratio from the data presented in Table 3 offer further support to the conclusion that oxygen fails to enhance radiosensitivity in treatment with small roentgen doses. Such estimations may be made by calculating the difference between the survival after irradiation with a dose in argon and with another dose in oxygen, and the corresponding ratio between the doses. Table 5 lists the survival ratios that correspond to roentgen doses chosen in such a way that their ratios are between 1 and 2. In considering such dose combinations, the survival appears always to be better after oxic than after anoxic treatment. The differences have varying degrees of statistical significance. Since an identical survival after oxic and anoxic treatment would indicate that the oxygen enhancement ratio equals the corresponding dose ratio, it may be concluded from the data in Table 5 that the oxygen enhancement ratio in these experiments is significantly lower than 2 and probably also lower than 1 2.

Split dose ratio under oxic and anoxic conditions. The effect of two exposures to radiation on the survival of ELD and Chinese hamster cells was investigated in relation to the effect of a single exposure to the same total dose combined with a subsequent sham irradiation. The time interval between two treatments

Table 5

Survival ratio after oxic and anoxic exposure to roentgen doses chosen in such a way that the dose ratio was between 1 and 2 — Data in Table 3 were used for the calculations

Exposure dose ratio in argon/in oxygen	Survival ratio Surviving fraction after treatment in oxygen/ surviving fraction after treatment in argon (^{e}log units)
$\frac{75}{50} = 1.5$	0.022
$\frac{100}{50} = 2.0$	0.062*
$\frac{100}{75} = 1.3$	0.015
$\frac{125}{75} = 1.7$	0.096
$\frac{150}{75} = 2.0$	0.111**
$\frac{125}{100} = 1.3$	0.041
$\frac{150}{100} = 1.5$	0.056
$\frac{150}{125} = 1.2$	0.022

*P (probability of no difference from zero) < 0.05 **P < 0.001

was 4, 12 or 18 hours. Irradiations were performed either in the presence or absence of oxygen. The results are illustrated in Fig. 4. Independently of cell strain and time interval between treatments the surviving fraction after two exposures was of a similar magnitude as after a single exposure to the equivalent dose when oxygen was absent. Thus, the split dose ratio defined by the ratio between the surviving fractions after a single and split doses was always close to unity. On the other hand, when irradiated in the presence of oxygen, double or treble the number of cells survived after split dose treatment than after corresponding single dose treatment. When the tests, performed with the same cell strain and with identical time interval between the exposures, are considered, the difference between the split dose ratio after oxic and anoxic treatment is significant ($p < 0.001$ or < 0.005).

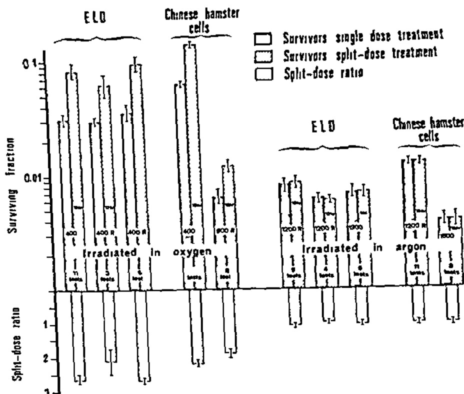


Fig 4 Survival of ELD and Chinese hamster cells after exposure in argon or oxygen to either a single roentgen dose or to same dose delivered in two equal fractions (upper scale) The time interval between the dose fractions was 4 12 and 18 hours as indicated in the respective columns during which period the cells were incubated in air and carbon dioxide The split dose ratio calculated from the difference between the survival after single and split doses are indicated (lower scale) Each column shows the mean with S E The mean plating efficiency varied between 47 to 70 and 91 to 96 per cent in the test with ELD and Chinese hamster cells respectively

A series of five repeat tests was performed to examine the possibility that the difference between the split dose ratios in argon and oxygen might have been due to some particular effect of the argon treatment before irradiation Chinese hamster cells were kept in argon for 15 minutes and within one minute there after irradiated in oxygen with either 700 R or 2×350 R at time intervals of 18 hours As a particular control cultures that were kept in oxygen for 5 minutes and subsequently irradiated with the same doses under identical conditions were employed The difference in the pretreatment with the two different gases does not affect either the survival or the split-dose ratio as indicated by the results in Table 6

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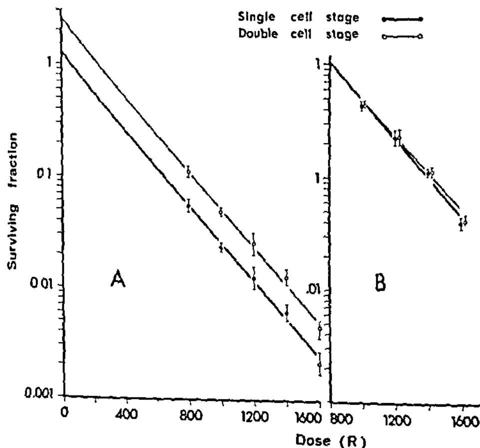


Fig 5 Survival of Chinese hamster cells after irradiation with increasing doses of roentgen rays in two exposures in argon *The first exposure followed the plating of the cells after a period of* 3 hours (single cell stage) *or* 21 hours (double cell stage) and was in all cases to 800 R. The second exposure followed the first exposure after a period of 18 hours (single cell stage) or 9 hours (double cell stage) to a dose varying *from* 200 *to* 800 *R* Between exposures the cells were incubated in air and carbon dioxide Means with 95 per cent confidence limits are shown from 5 repeated tests with a mean plating efficiency of 93 ± 5 per cent Left part of figure (A) illustrates survival relative *to the unirradiated* cells right part (B) the survival relative to the survivors of 800 R treatment

a single cell in a sister pair is $2 \times (1 - 0.06) \times 0.06 = 0.1152$ This suggests that the overwhelming majority ($\frac{0.1152}{0.1152 + 0.0036} = 97\%$) of the survivors in the population exposed to an initial radiation with 800 R after cell division will consist of single cells and not of sister *pairs* i e the cell multiplicity will be 1 when the second radiation dose is applied

Table 6

Effect of pretreatment with argon or oxygen on the survival of Chinese hamster cells exposed to one irradiation with a single or split doses

Treatment before irradiation	Plating efficiency ±S. E. (%)	Exposure dose in oxygen (R)	Surviving fraction (%)		Split dose ratio	
			Mean ± S. E. (^{e}log units)	Arithmetic value of mean	Mean ± S. E. (^{e}log units)	Arithmetic value of mean
15 min in argon	85 ± 5	700	−0.149 ± 0.115	0.86	0.830 ± 0.151	2.29
		2 × 350	0.681 ± 0.127	1.98		
5 min in oxygen	84 ± 5	700	−0.173 ± 0.126	0.84	0.781 ± 0.100	2.18
		2 × 350	0.608 ± 0.130	1.84		

Survival regression after irradiation with split doses. The survival regression after split dose treatments was studied by double exposures of the hamster cells to increasing total roentgen doses. The first exposure was always to a dose of 800 R, the second one to a dose varying from 200 to 800 R, all exposures being made in argon. In each of the five repeat tests, the cells were in two different stages of growth when irradiated for the first time. In one case, the first dose was given 3 hours after plating of the cells, followed by the second dose after an interval of 18 hours. In the second case, the cells were irradiated with the first dose 21 hours after plating, and with the next dose after a further interval of 9 hours. These time intervals were chosen from two previous observations. On the one hand, cells that have been irradiated 3 hours after plating, will not divide for 18 hours. On the other hand, if the first irradiation is made 21 hours after plating, the cells will have completed one division and the population will have doubled and consist of sister pairs. In the latter instance, the survivors will not have completed a second division within a further period of 9 hours.

The survival of the irradiated cells in relation to unirradiated controls is illustrated in Fig. 5 A. The numerical value of the survival parameters calculated by least square analysis are presented in Table 7. The slope constants are almost identical whether the exposure to radiation was made before or after the first division of the cells. In contrast, the extrapolation number of the regression line at a zero dose is close to unity when the cells were treated before division, and has a value between 2 and 3 when the cells were treated after division.

Considering that the probability of a cell surviving irradiation with 800 R, used as the first dose, is 0.06 (cf. Figs. 2 and 5 A), it can be calculated that the probability of both cells in a sister pair surviving is $0.06^2 = 0.0036$, and that of

et coll 1963a) The role of the inherent sulphydryls in influencing intrinsic radiosensitivity is further supported by the finding that clone a with the same sensitivity as ELD has a thiol concentration of the same magnitude (REVESZ et coll 1963a) The increased radioresistance of ELT with a sulphydryl concentration of the same magnitude as ELD (CASPERSSON & REVESZ 1963 REVESZ et coll 1963a) can be associated with the duplicated genome of this strain as discussed in other reports (GLAS & REVESZ 1963 REVESZ et coll 1963b REVESZ & LITTBRAND 1964) The possible necessity of inactivating a larger number of sensitive sites has probably accounted for the radioresistance in such a case However since the extrapolation number of the ELT survival curve is similar to the extrapolation of ELD whether oxic or anoxic irradiation was used (cf Fig 2) some other factors in addition to or instead of an increased target number may also play a role The observations of SILINI & HORNSEY (1962) and BERRY (1963) on an increased extrapolation number of tetraploid tumors in comparison to diploid ones may not be appropriate in this context The cell lines in their tests were not sublines of each other and the change in the extrapolation number may be associated therefore to some difference between lines other than ploidy

The difference in radiosensitivity demonstrated between the cell strains irradiated in anoxia was shown to be reduced to statistically insignificant variations when the exposures to roentgen radiation was made in oxygen This implies that the oxygen enhancement ratio is different for the different cell strains Statistical analysis of the data indicated that the differences in the oxygen enhancement ratio were significant (Table 3) A reduction of the sensitivity differences in this gas as compared to anaerobic conditions has been previously noted also when the cell strains used in the present tests were investigated in vivo (REVESZ et coll 1963b REVESZ et coll 1967) Similar observations were reported by SILINI & HORNSEY (1962) in their in vivo tests The relationship between the values of oxygen enhancement ratios of diploid and tetraploid cell lines used by these authors (2 7/3 1) was similar to the relationship observed in our case 2 48/2 83

The oxygen effect can apparently mask variation in cellular sensitivity whether due to a difference in ploidy or to the SH content If a proportionately increased sulphydryl concentration accounts for the relative resistance of some cell lines in anoxia then the disappearance of this resistance in oxygen would be in agreement with the observation of a considerable decrease of the sulphydryl protection in an oxygenated medium (DEWEY 1963 HOWARD FLANDERS 1960 SHALEK & SMITH 1968) It can also be argued that the correlation between radiosensitivity and ploidy observed when treatment is made in anoxia may imply the principally genetic character of the radiation injury to the cell The decrease or abolition of such a correlation when treatment is made in oxygen suggests the prevalence of

Table 7

Numerical value of the survival parameters in the tests illustrated in Fig. 5

Time interval between plating and 1st irradiation	Time interval between 1st and 2nd irradiation	Survival of irradiated cells relative to unirradiated controls				Survival of irradiated cells relative to survivors of 800 R			
		Slope constant of survival regression Mean ± S.E. (^{e}log units)	D_0 (R)	Extrapolation number*		Slope constant of survival regression Mean ± S.E. (^{e}log units)	D (R)	Extrapolation number**	
				Mean ± S.E. (^{e}log units)	Arit. value of mean			Mean ± S.E. (^{e}log units)	Arit. value of mean
3 hours	18 hours	0.390 ± 0.018	256	0.232 ± 0.096	1.26	0.393 ± 0.027	254	0.031 ± 0.129	1.03
21 hours	9 hours	0.387 ± 0.018	259	0.906 ± 0.076	2.47	0.389 ± 0.02[illegible]	257	0.023 ± 0.120	1.0[illegible]

*Calculated from the intersection of the statistical regression line with the ordinate at zero dose.
**Calculated from the intersection of the statistical regression line with the ordinate at 800 R.

In Fig. 5 B the survival of the irradiated cells relative to the survivors of a treatment with 800 R is illustrated. The numerical value of the survival parameters, calculated by least square analysis is presented in Table 7. The slope constants are, again, similar if the data obtained in tests with cells before and after division are considered. However, in this situation the extrapolation numbers are again similar and close to unity in both cases.

Discussion

Slope of survival regression. The results indicate that under anoxic conditions significant differences in radiosensitivity exist between cell strains selected from the same original tumor cell population. It may be concluded that in agreement with the wide intercellular variation of different characteristics (Harris 1964), neoplastic cell populations may also vary with regard to the radiosensitivity of their cellular components. It may be calculated from the relationship between the slope constants that the sensitivity of clone g and I LT is 24 and 19 per cent less, respectively, than the sensitivity of the original ELD. The decreased sensitivity of clone g may be attributed to the protective effect of the increased cellular concentration of low molecular sulphydryls, which has been estimated to be 24 per cent higher in this clone than in ELD (Caspersson & Revesz 1963, Revesz

et coll 1963a) The role of the inherent sulphydryls in influencing intrinsic radiosensitivity is further supported by the finding that clone a with the same sensitivity as ELD has a thiol concentration of the same magnitude (REVESZ et coll 1963a) The increased radioresistance of ELT with a sulphydryl concentration of the same magnitude as ELD (CASPERSSON & REVESZ 1963 REVESZ et coll 1963a) can be associated with the duplicated genome of this strain as discussed in other reports (GLAS & REVESZ 1963 REVESZ et coll 1963b REVESZ & LITTBRAND 1964) The possible necessity of inactivating a larger number of sensitive sites has probably accounted for the radioresistance in such a case However since the extrapolation number of the ELT survival curve is similar to the extrapolation of ELD whether oxic or anoxic irradiation was used (cf Fig 2) some other factors in addition to or instead of, an increased target number may also play a role The observations of SILINI & HORNSEY (1962) and BERRY (1963) on an increased extrapolation number of tetraploid tumors in comparison to diploid ones may not be appropriate in this context The cell lines in their tests were not sublines of each other and the change in the extrapolation number may be associated therefore to some difference between lines other than ploidy

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some non genetic mechanism operative in the presence of this gas and involving structures and systems other than the genetic apparatus alone This conclusion is in agreement with the postulate based on observations made in experiments with micro organisms that DNA is more heavily involved in killing by roentgen radiation under anoxic as compared to aerobic conditions (ALPER 1963)

Extrapolation numbers The extrapolation number (n) of the survival curves of ELD and its substrains, as well as the Chinese hamster cells, were similar and had values around 2 when irradiations were administered in the presence of oxygen, and values close to unity when the irradiations were given in the absence of the gas The relatively low value of n of the oxic survival curves may be attributed to the fact that the cells used in the survival tests were derived from 6 to 8 day old cultures, i e were in a stationary phase of growth HAHN (1968) reported that the survival curve of such cells extrapolate to lower values than cells derived from cultures in the phase of exponential growth

The extrapolation number to unity found throughout in the tests when cells were irradiated under anaerobic conditions, was found (RÉVÉSZ & LITTBRAND 1969) to be independent of the growth phase of the cultures from which the cells were derived This is also indicated by the experiments (cf Table 7) in which the cell population used has completed one division after plating and before irradiation The cells in such a population may be regarded as having full metabolic activity and being in an early phase of exponential growth When the survival regression of such cells was established the extrapolation number was unity (cf Fig 5 B), as in the case of cells which were, as a routine, derived from cultures in their plateau phase and which had probably an impaired metabolism

The difference between the extrapolation numbers when irradiation was made in argon and oxygen as well as the difference in the oxygen enhancement ratio of closely related cell lines suggest that oxygen does not act as a simple, dose modifying factor It may be inferred that, in addition to a quantitative difference there is also a qualitative difference in the lethal damage caused by irradiation in the prescence and absence of oxygen In view of recent findings that indicate the oxygen dependency of the recovery process it is conceivable that the difference concerns the repair of the radiation damage (LITTBRAND & RÉVÉSZ 1969) As an alternative explanation, the extrapolation to unity and a corresponding nearly exponential dose effect relationship may denote that a single damaging event is sufficient to abolish the reproductive capacity of anoxic cells The dose effect relationship after anoxic treatment with the sparsely ionizing roentgen rays, is indeed similar to that observed after treatment with densely ionizing radiations (BARENDSEN 1962 BARENDSEN et coll 1963, DEERING & RICE

1962) On the other hand an extrapolation number larger than unity noted after aerobic treatment may be regarded as indicating that more than one damaging event of the same or a different kind (genetic or non genetic) must be accumulated for any lethal effect

The crossing of the extrapolated survival regression of the oxic and anoxic cells (Fig 2) implies that the effect of oxygen in enhancing sensitivity varies with the magnitude of the exposure dose In the dose range before the crossing a negative enhancement i e a protecting effect may prevail Experimental evidence for such a protection was not obtained in the tests performed in 100 per cent oxygen with either single exposure doses in the range of 50 to 125 R or with multiple doses of 100 R though this latter treatment may be particularly suitable for discovering small differences in survival The differences between the survival of oxic and anoxic cells were always insignificant and an oxygen enhancement ratio close to unity was indicated With increasing doses after the crossing the oxygen enhancement ratio could be expected to increase from unity toward a maximal value defined by the ratio between the slope constant of the oxic and anoxic survival regressions (REVESZ & LITTBRAND 1967a) This expectation received experimental proof in tests in which the survival curve of oxic cells was determined after exposure to doses between 200 and 600 R and that of anoxic cells after exposure to doses between 800 and 1 800 R It is evident that the enhancement of radiosensitivity by oxygen is a function also of the exposure dose and is therefore more complex than expressed by the formula proposed by HOWARD FLANDERS & ALPER (1957) A more detailed discussion of this problem has been published elsewhere (LITTBRAND & REVESZ 1969)

Recovery and the oxygen effect In considering the relationship between the split dose ratio and the extrapolation number (ELKIND & SUTTON 1960) the split dose ratio around 2 and 1 found in the oxic and anoxic experiments respectively is in agreement with the theoretical expectations It has been shown that a summation of survival curves with diverse extrapolation numbers (DITTRICH 1960) or slope constants (POWERS & TOLMACH 1963) may result in a common composite curve with a decreased extrapolation number The close agreement between extrapolation number and split dose ratio excludes this possibility, i e the heterogeneous response of the cell population as an explanation of the findings The recovery observed when cells were pretreated in argon and subsequently irradiated in oxygen (Table 6) also contradicts the argument that such pretreatment is in some way responsible for the impaired recovery when irradiation is made anoxically The data of the different experiments are thus concordant in indicating that recovery requires oxygen The results of experiments in which recovery was studied at varying oxygen tensions strongly suggest that

oxygen is required as the energy source for the recovery process (LITTBRAND & REVESZ 1969).

Since the cells in the experiments immediately after exposure to radiation in anoxia were put under aerobic conditions, it may also be concluded that post-treatment access to oxygen does not restore recovery. Recovery is, indeed, dependent upon the presence of oxygen during or very shortly after the radiation treatment. It has been argued (LITTBRAND & REVESZ 1969) that the damage induced by radiation may be recoverable only during a limited time period. If oxygen is present during the critical period, the recovery process may begin and continue whilst its energy requirement is satisfied.

Oxygen may play a dual role in determining the reaction of cells to radiation: on the one hand, it may sensitize the cells in enhancing the radiation damage and, on the other hand, it may protect them in permitting recovery. Apparently, in the dose range around the crossing of the extrapolated survival curves, the protecting effect can wholly compensate for the injury due to the sensitizing effect. With larger doses, the protecting effect has a decreasing significance in relation to the sensitizing effect. The relative importance of the two antagonistic effects has been shown to be dependent also upon the oxygen concentration (REVESZ & LITTBRAND 1967b, LITTBRAND & REVESZ 1969). As an example, a concentration of 0.5 μM oxygen in the medium permits the development of almost maximal recovery while it sensitizes only slightly (LITTBRAND & REVESZ 1969). This indicates that an oxygen enhancement ratio close to some known maximal value cannot be simply taken as evidence for the prevalence of total anoxic conditions in a certain experimental system.

Present observations in relation to earlier studies. The results presented in this paper are in full agreement with preliminary observations (LITTBRAND & REVESZ 1964, ROBINSON & REVESZ 1961). They also confirm the data obtained in experiments in which the effects on radiosensitivity of cysteamine, triacetoneamine-N-oxyl or oxygen in varying concentrations were studied on radiosensitivity; cells irradiated similarly in anoxia or 100 per cent oxygen being used as controls (LITTBRAND & REVESZ 1969, to be published, REVESZ & LITTBRAND 1970). The findings reported by PHILLIPS (1968) are also in agreement with our observation on an oxygen dependent extrapolation number. This author irradiated bone marrow cells in vitro in the presence of oxygen or nitrogen. In subsequent in vivo tests with the spleen colony technique, the aerobic survival curves extrapolated to 1.5 while the anaerobic curves extrapolated close to unity. In an in vitro system with a long gassing equilibrium time, HALL et coll. (1966) found a reduction of the extrapolation number to unity when the radiation survival of HeLa cells was studied after storage in severe nitrogen hypoxia for

36 hours. A 6 hour storage resulted in no reduction of the extrapolation number. In in vitro experiments with L cells HUMPHREY et coll. (1963) noted a reduction of the extrapolation number from 12 to 3 when cells were irradiated under hypoxic conditions in nitrogen. Under conditions similar to those of the present material a 20 second flushing with nitrogen before irradiation reduced the extrapolation number of the survival curve of Chinese hamster cells from 3.8 to 2.4 (ELKIND et coll. 1968). In contrast, when these cells were irradiated in a different in vitro system the extrapolation number in nitrogen was similar to the aerobic one. Experiments with HeLa cells in vitro gave similar results in that the extrapolation number failed to change whether oxygen or nitrogen was the gas phase (MAS et coll. 1967).

In a general agreement with the present results no repair of the radiation damage was noted when ascaris eggs were treated with fractionated radiation doses under hypoxic conditions (LIECHTI 1929). A decreased split dose ratio was calculated also by HALL & CAVANAGH (1967) in experiments with *Vicia faba* when irradiation was applied in nitrogen instead of aerobic conditions. In contrast in split dose experiments with Chinese hamster cells exposed in nitrogen recovery was demonstrated by ELKIND et coll. (1965). When cellular survival curves were determined in experiments in vivo extrapolation numbers dependent (BELLI et coll. 1967, PHILLIPS 1968, VAN PUTTEN & KALLMAN 1968) or independent (HEWITT & WILSON 1961, REINHOLD 1966) of the presence of oxygen during the irradiation were reported.

A common interpretation of the contradictory results is difficult at present. Apart from differences in the cellular material and experimental conditions the statistical analysis of the significance of the data is often incomplete and uncertainty often concerns also the degree of hypoxia. The interpretation of split dose experiments in vivo is in addition still complicated by the possible repopulation and re-oxygenation of the irradiated tissue during the time interval between the treatments. While recovery of hypoxic cells in vivo was reported by many investigators (BERRY et coll. 1967, FOWLER et coll. 1965, HORNSEY 1967) recent carefully conducted experiments also indicated that the recovery of such cell may be substantially decreased (ELKIND et coll. 1968, BELLI et coll. 1967) or absent (PHILLIPS & HANKS 1968). In a recent paper SUIT & URANO (1969) reported that acutely hypoxic mammary carcinoma cells repaired damage rapidly and extensively. In contrast chronically hypoxic cells were less able to repair radiation injury.

The danger of hypoxic cells. Considering the varying oxygen gradients prevailing in human tumors (THOMLINSON & GRAY 1955) and the finding (LITTBRAND & REVESZ 1968) that cells can survive for a prolonged period of time even when

less than 0.004 μM O_2/l is available in the medium, the present results and conclusions may carry some radiotherapeutic implications. While well oxygenated portions of a tumor and normal tissues with unimpaired blood supply will suffer a larger initial radiation damage than the anoxic tumor cells, the oxic cells can probably recover but the damage to anoxic cells may not be repaired. Fractionated irradiation may therefore result in a greater additivity of the injury in the anoxic cells than in oxygenated cells. Fractionation of the radiation dose can be expected to reduce the anoxic protection ratio which, with approximately small doses, may have a value even less than 1. The potential danger of surviving therapeutic radiation would seem to be greatest with the hypoxic rather than anoxic cells since the former will not only suffer less injury but also have the capacity of repairing a part of the damage (LITTBRAND & REVESZ 1969). Transfer of cells from the initially anoxic to the hypoxic compartment as a result of partial re-oxygenation may constitute a particular hazard with regard to the success of therapy.

Conclusions

Cultures of Chinese hamster cells, an Ehrlich ascites tumor and three of its substrains were exposed to roentgen radiation in oxygen or argon with an oxygen content less than 2 ppm. Significant differences in radiosensitivity between the ascites tumor and the substrains were demonstrated when irradiation was administered in argon. In oxygen the radiosensitivities were similar implying differences in the oxygen enhancement ratio between different cell strains. The survival curves after anoxic irradiation with either single or split exposure doses were exponential with extrapolation numbers to a zero dose close to unity. In oxygen the survival curves were sigmoid and the extrapolation numbers around 2. This difference between the extrapolation numbers implies a crossing of the survival curves corresponding to a dose range where theoretically no oxygen enhancement of sensitivity can be expected to occur. Experiments performed with single exposures to 125 R or less and with three exposures to 100 R, proved the correctness of this expectation and indicated that survival is independent of the presence or absence of oxygen in this dose range.

The split dose ratios calculated from the survival after single exposure and two exposures to the same dose separated by an interval of 4, 12 or 18 hours agreed well with the extrapolation numbers and were around 2 and close to 1 when irradiations were given in the presence and absence of oxygen, respectively.

Differences in the cellular concentration of non-protein bound sulphydryl groups and the ploidy grade were considered to explain the differences in the anoxic radiosensitivity between the Ehrlich ascites tumor cells and its substrains. The role of oxygen in the expression of radiation damage was considered as a

dual one acting as an essential modifying agent to both the process of sublethal recovery and lethal damage

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SUMMARY

The survival parameters of different cell line were studied after exposure to roentgen radiation Oxygen affected both production and repair of radiation damage The extent of these processes varied in the different cell lines

ZUSAMMENFASSUNG

Die Uberlebensparameter verschiedener Saugetier Zell Linien wurden nach Rontgen bestrahlung untersucht Sauerstoff beeinflusste sowohl die Bildung als auch die Reparation des Strahlenschadens Das Ausmass dieser Prozesse variierte in verschiedenen Zell Linien

RÉSUMÉ

L'auteur a etudie les parametres de survie de differentes lignees cellulaires de mammiferes apres exposition aux rayons de roentgen L'oxygene a une influence aussi bien sur l'apparition que sur la réparation des radio lesions L'importance de ces processus est variable dans les differentes lignees cellulaires

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CONTROLE DOSIMETRIQUE EN BRACHYCURIETHERAPIE PAR LES ISODOSES 'ESCARGOT'

par

M. SCHLIENGER, J. C. ROSENWALD, M. MICLUTIA, R. QUINT et B. PIERQUIN

Nous présentons une modification pratique du calcul de la dose en brachycuriethérapie par iridium 192. Il s'agit d'une adaptation de notre technique sur points, systématisée en 1960 (PIERQUIN et coll. 1960 et 1962) et modifiée une première fois en 1964 par l'emploi des isodoses circulaires standard (PIERQUIN et coll. 1964).

Rappel des isodoses circulaires standard. Ce contrôle dosimétrique s'associe à l'étude prévisionnelle (PIERQUIN et coll. 1968) établie pour chaque malade. Son but est de calculer, après la mise en place du dispositif radifère, la répartition de la dose en différents points à l'intérieur et autour de ce dispositif formé de lignes radio-actives continues et parallèles. Cette dosimétrie est établie dans un ou plusieurs plans perpendiculaires aux lignes radio-actives. Ce ou ces plans de coupe sont obtenus par tomographie ou par reconstruction. Les images des fils ou aiguilles apparaissent sur la tomographie ou le schéma de reconstruction sous forme de points. Ces points et les contours des structures anatomiques reconnues sont dessinés sur un calque.

Soumis à la Rédaction le 25 Juin 1969

Des isodoses circulaires standard gravées sur des tampons encreurs en caoutchouc ont imprimées sur le calque autour de chaque point. Elles ont été calculées à partir d'un facteur k arbitraire de valeur 1 R/h 1 mCi/cm pour des doses de 10—4—2—1—0,6—0,4—0,2 R. Un jeu de dix tampons permet de disposer d'isodoses standard pour des longueurs radio-actives de 1—2—3—4—5—6—7—8—10—12 cm.

Par recoupement entre ces différentes isodoses circulaires, la dose peut être calculée en n'importe quel point du plan de coupe. En quelques minutes, on peut ainsi calculer la dose de base et la dose de référence, et finalement dessiner toutes les isodoses désirables.

Imperfections des isodoses circulaires standard. Cette méthode de calcul de la dose comporte quelques imperfections énumérées ci-dessous.

1. Les isodoses sont calculées en roentgens et non en rads.
2. Leur universalité ne permet pas d'introduire le coefficient d'atténuation dans les tissus, coefficient variable d'un isotope à un autre.
3. La lecture des isodoses devient difficile lorsque le dispositif radifère est trop serré et trop nombreux : des fautes d'identification deviennent possibles.
4. Inversement, les espacements entre les isodoses d'un même tampon sont relativement importants, d'où des risques d'erreur d'interpolation dans le calcul de la dose en tel ou tel point du volume cible (surtout dans l'espace compris entre l'isodose 10 et l'isodose 4).
5. Un léger décentrement des isodoses (de l'ordre du millimètre) par rapport au point de section de la ligne radio-active est pratiquement inévitable au moment du tamponnement.

Toutes ces imperfections peuvent aboutir à des erreurs de calcul de l'ordre de 10 %. Elles se compensent bien souvent, mais elles laissent une marge d'incertitude non négligeable.

Les isodoses 'escargot'

A l'encontre des isodoses tampons centrées sur les différents points de section des lignes radio-actives, ces isodoses escargot sont placées sur chaque point tumoral étudié pour la recherche de la dose de base, de la dose de référence ou de toute autre dose. Elles n'intéressent qu'un seul radio-élément, en l'occurrence l'iridium 192, ce qui permet d'introduire dans le calcul le coefficient d'atténuation et la valeur de la constante radio-active (facteur k). Elles ont été dessinées à partir d'un calcul sur ordinateur à l'aide d'un traceur de courbes.

Pratiquement, il s'agit d'un jeu d'isodoses inscrites sur trois feuilles transparentes en rhodoïd. La première feuille contient les isodoses pour des longueurs

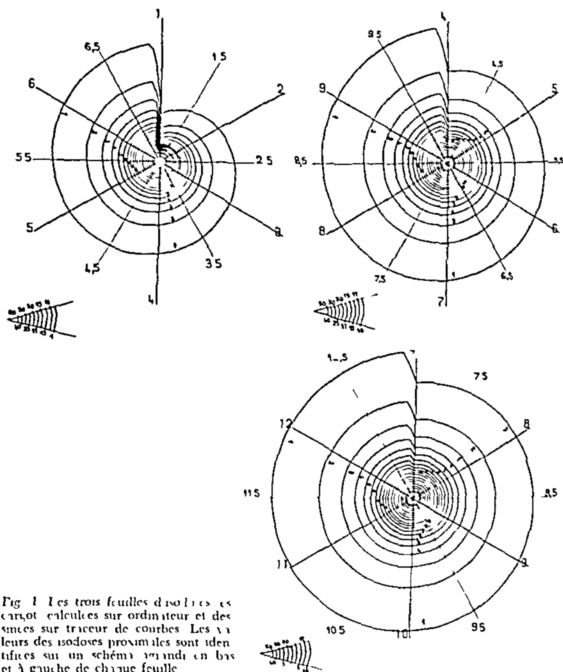


Fig. 1. Les trois feuilles d'isodoses escargot calculées sur ordinateur et dessinées sur traceur de courbes. Les valeurs des isodoses proximales sont identifiées sur un schéma agrandi en bas et à gauche de chaque feuille.

radio actives comprises entre 1 et 6,5 cm. La seconde feuille va de 4 à 9,5 cm et la troisième feuille de 7 à 12,5 cm. Les lignes radio actives sont donc étudiées de 0,5 en 0,5 cm. Elles se disposent en rayons de roue autour du point central. De cette façon, en choisissant une seule de ces feuilles par leur chevauchement on peut couvrir, dans la plupart des cas, toutes les longueurs utilisées dans le



Fig 2 Six fils d iridium 192 de 3 cm de longueur implantes dans un carcinome epidermode de la langue mobile Tomographie transversale de controle Agrandissement 1 33

dispositif radifere considere que ces longueurs soient egales a un nombre entier de centimetres ou non

Ces differentes isodoses sont au nombre de 19 a savoir 50—40—30—25—20—17—15—13—11—10—9—8—7—6—5—4—3—2—1 rad/heure pour une activite de 1 mCi cm (iridium 192) L inscription continue pour chaque valeur et entre les differentes longueurs radio-actives determine des isodoses de forme helicoidale d ou le sobriquet d isodoses escargot (Fig 1) Ces isodoses sont bien entendu agrandies d un facteur 1 33 qui correspond au coefficient de grandissement des tomographies

Le calcul s effectue de la façon suivante

Le calque de la tomographie ou de la reconstruction agrandie du facteur 1 33 est place sur une plaque de plexiglas perforee en son centre

La feuille adequate d isodoses escargot est place sur ce calque en solidarisant son point central avec le point tumoral etudie a l aide d une epingle simple perforant simultanement la feuille d isodoses le calque et la plaque de plexiglas

En faisant tourner la feuille d isodoses autour de son point central on met successivement en coincidence le point de section de chaque ligne radio active apparaissant sur le schema avec le rayon de la feuille d isodoses qui correspond

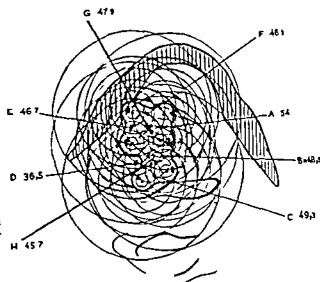


Fig 3 Dosimetrie de l implantation (voir la figure 2) par la technique sur points avec tampons en isodoses circulaires standard Isodose de reference 40 R/heure pour 1 mCi/cm

a sa longueur En quelques dizaines de secondes on peut ainsi totaliser la dose recue de l ensemble du dispositif radifere au niveau du point tumoral ou peri tumoral etudie

Nous conseillons de calculer tout d abord la dose au niveau du ou des differents points de *la dose de base* puis de dessiner la limite du volume cible souhaite, enfin de calculer quelques points tests sur cette limite afin de determiner la position definitive de *l isodose de reference* au niveau de laquelle sera calculee la dose tumorale totale Avec un peu d experience, ce controle dosimetrique peut etre realise en quelques minutes (Figs 2—4)

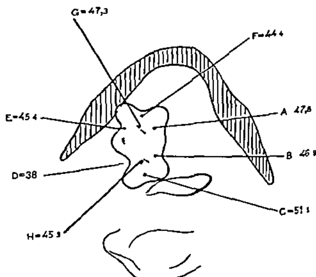
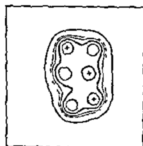


Fig 4 Dosimetrie de la meme implantation (voir les figures 2 et 3) par la technique sur points avec des isodoses escargot Isodose de reference 40 rad/heure pour 1 mCi/cm Dans ce cas considere la dose calculee en R (figure 2) aurait conduit pour un meme volume cible apparent à une surevaluation par rapport à la dose calculee en rad (figure 3)



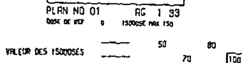


Fig 5 Dosimetrie de la meme implantation (voir les figures 2 -4) par ordinateur a et traceur de courbes L isodose de reference 40 rad/heure pour 1 mCi/cm est superposable à celle dessinee à partir des isodoses escargot

Discussion

Ces isodoses escargot comportent plusieurs avantages

1 La precision du dessin grace au calcul de ces isodoses par ordinateur leur inscription directe par traceur de courbes

2 Le grand nombre de valeurs disponibles tant en longueurs radio-act qu en isodo es les interpolations sont de ce fait reduites a des intervalles ne geables

3 La large surface de ces feuilles d isodoses permettant d inclure des isod de faible valeur a grande distance du point etudie Ces isodoses escargot donc utilisables pour de grands volumes-cibles

4 La clarte de la lecture malgre le grand nombre d isodoses le calque comportant que les marques des points de section des lignes radio-actives et points tumoraux ou peri tumoraux etudies

5 Le faible investissement de ce materiel et son absence d encombrement dix tampons de l ancien systeme se trouvant remplaces par trois feuilles de r doid

Quelques reserves sont cependant a formuler

1 Le resserrement des isodoses de valeur elevee implique une lecture att tive sous peine d erreurs d interpolation

2 La delimitation de l isodose de reference (ou de toute autre isodose a l terieur ou autour du volume cible) n est plus guidee par le recoupement isodoses circulaires des tampons il faut apprendre à dessiner l isodose de referen dans le vide a partir de quelques points judicieusement calcules sur la lim de ce que l on prevoyait etre dans l enveloppe du volume traite

3 Le facteur d agrandissement 1 33 necessite de corriger les mensuratio directe en cas de reconstruction

tomographe. (Il reste toujours possible de faire dessiner les isodoses escargot sans agrandissement, mais la lecture sur le calque devient alors très difficile, les isodoses étant trop rapprochées. L'agrandissement est en fait une nécessité pour obtenir une lecture claire.)

4. Ces isodoses escargot n'ont été calculées que pour l'iridium 192. Cette limitation, indispensable pour la précision de la dosimétrie, ne peut plus être maintenant considérée comme un handicap du fait de la généralisation de l'emploi de l'iridium 192 en brachycuriethérapie avec lignes radio-actives parallèles (bien entendu, il est loisible de calculer, selon la même méthodologie, des isodoses escargot pour d'autres radio-éléments).

RÉSUMÉ

Les isodoses escargot permettent de simplifier le contrôle dosimétrique en brachycuriethérapie par iridium 192 avec lignes radio-actives parallèles. Grâce au calcul sur ordinateur *et au dessin sur traceur de courbes, elles permettent d'effectuer une dosimétrie avec précision accrue.* Leur prix de revient et leur encombrement négligeables permettent de les utiliser dans tous les centres de radiothérapie disposant d'iridium 192.

SUMMARY

The dosimetric control in brachycurietherapy with ididium 192 and parallel radioactive lines can be simplified determining escargot isodoses. Dosimetry can in this way be carried out with great precision by means of computer calculation and tracing of curves. The costs and space requirements are negligible and the method can be used in all radiotherapeutic centres where iridium 192 is employed.

ZUSAMMENFASSUNG

Escargot Isodosen erlauben eine Vereinfachung der Kontrolle der Dosimetrie bei der Brachycurietherapie mit Iridium 192 und radioaktiven parallelen Linien. Es ist möglich die Dosimetrie mit verbesserter Genauigkeit durch Berechnungen mit einem Computer und Aufzeichen von Kurven zu planieren. Die Kosten und der Platzaufwand sind zu vernachlässigen und die Anlage kann von allen radiotherapeutischen Zentren die über Iridium 192 *verfügen verwendet werden.*

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RADIATION THERAPY AND CURE IN HODGKIN S DISEASE

by

K. Musshoff, W Oehlert W Hamann and A Nuss

Malignant lymphogranulomatosis (Hodgkin s disease) is usually regarded as cured if full remission following primary treatment lasts nine years or longer (Musshoff & Boutis ref 11) Primary treatment may be interpreted as including a second treatment soon after the first recurrence at the margins of the primary lesion or in a neighbouring lymph node

The *purpose of this* communication is first to describe the general clinical conditions *prior to* 1960 *upon* which the cures were based (consideration must therefore be given to the *fact that* the diagnostic examinations of those years were usually confined to the *establishment of the* disease and that further differentiating laboratory tests were generally *not conducted*) second to discuss the histologic diagnosis and its classification (Lukes & Butler ref 9) and third to suggest the methods of therapy by which a cure may best be achieved

Material This consists of a series of 28 patients from a total of 393 with Hodgkin disease treated primarily during the period 1948—1967 and classed as cured The original slide preparations for sixteen of these twenty eight patients

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tomographie (Il reste toujours possible de faire dessiner les isodoses 'escargot sans agrandissement, mais la lecture sur le calque devient alors tres difficile, les isodoses etant trop rapprochees L'agrandissement est en fait une necessite pour obtenir une lecture claire)

4 Ces isodoses escargot n'ont ete calculees que pour l iridium 192 Cette limitation, indispensable pour la precision de la dosimetrie, ne peut plus etre maintenant consideree comme un handicap du fait de la generalisation de l em ploi de l iridium 192 en brachycurietherapie avec lignes radio actives paralleles (bien entendu, il est loisible de calculer, selon la meme methodologie, des iso doses 'escargot pour d autres radio-elements)

RÉSUMÉ

Les isodoses escargot permettent de simplifier le controle dosimetrique en brachycurie therapie par iridium 192 avec lignes radio actives paralleles Grace au calcul sur ordinateur et au dessin sur traceur de courbes elles permettent d effectuer une dosimetrie avec pre cision accrue Leur prix de revient et leur encombrement negligeable permettent de les utiliser dans tous les centres de radiotherapie disposant d iridium 192

SUMMARY

The dosimetric control in brachycurietherapy with ididium 192 and parallel radioactive lines can be simplified determining escargot isodoses Dosimetry can in this way be carried out with great precision by means of computer calculation and tracing of curves The costs and space requirements are negligible and the method can be used in all radiotherapeutic centres where iridium 192 is employed

ZUSAMMENFASSUNG

Escargot Isodosen erlauben eine Vereinfachung der Kontrolle der Dosimetrie bei der Brachycurietherapie mit Iridium 192 und radioaktiven parallelen Linien Es ist moglich die Dosimetrie mit verbesserter Genauigkeit durch Berechnungen mit einem Computer und Aufzeichen von Kurven zu planieren Die Kosten und der Platzaufwand sind zu vernach lassigen und die Anlage kann von allen radiotherapeutischen Zentren die uber Iridium 192 verfugen verwendet werden

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Table 2

Life expectation in malignant lymphogranulomatosis — Influence of histologic type on survival time according to LUKES & BUTLER *(1966) — Freiburg patient data 1948—1967*

Histologic type	Numbers	Survival rates in per cent		
		3 year	5 year	10-year
Lymphocytic and histiocytic nodular	11	72 8	46 ?	46 2
Lymphocytic and histiocytic diffuse	13	76 9	68 8	58 2
Nodular sclerosis	88	74 5	56 1	36 4
Mixed	40	49 8	36 6	30 5
Diffuse fibrosis	21	43 5	43 5	11 6
Reticular	20	48 1	30 1	30 1
Total	193	63 8	49 0	33 7

volvement on one side of the diaphragm without and with localized organ extension)

The cured patients belong to both clinical forms without as well as with symptoms or signs of active disease the number of patients without signs is only slightly higher than those with signs (Table 1)

Histology in cured malignant lymphogranulomatosis As already stated the original slide preparations from sixteen of the twenty-eight cured patients were evaluated and a histologic report was available for a further four patients Of the remaining eight patients either the report or the slide preparation was insufficient or lymph node punctates had been made Twenty patients thus remain for classification according to the histologic criteria of LUKES & BUTLER (ref 9)

The histologic appearances of the lymphocytic and histiocytic forms consist of a distinct predominance of lymphocytes and histiocytes and only a few Hodgkin and Sternberg giant cells. According to LUKES et coll (ref 10), as well as to the similar Freiburg classification these forms carry the best prognosis (Table 2)

The diffuse fibrosis and reticular forms in which a marked reduction in lymphocytes and a predominance of Hodgkin and Sternberg giant cells with varying degree of fibrosis occur have a poor prognosis

The prognosis in the nodular sclerosis form is almost as good as in that of the first two groups All other forms are regarded as mixed forms in which the prognosis is indifferent

Table 3 indicates that most of the twenty classifiable patients considered as potentially cured belonged to the nodular sclerosis group approximately 40 %

Table 1

Distribution of 28 cured patients (13 males and 15 females) according to stage and clinical form of Hodgkin's disease

Clinical form	Stages according to the Rye classification				Number of patients
	I	II	III	IV	
A	6	10	—	—	16
B	3	5	—	4	12
Total	9	15	—	4	28

were evaluated. A classification by the LUKES method was made in four of six patients after the detailed report of histologic investigation, while in a further six patients the diagnosis had been cytologically verified.

Results

The treatment of the 393 patients has been discussed in detail elsewhere (MUSSHOFF & BOUTIS ref. 12, 13, 14). The 5 year survival rate of this series amounted to 48.6 %, the 10 year survival rate to 29.3 %, while the 15 and 20 year survival rates were each 23.1 %.

Spread, signs, histologic types and treatment of the twenty eight cured patients are presented in Tables 1, 3 and 4. The clinical stage was evaluated according to the classification proposed at the Lymphogranulomatosis Symposium at Rye, New York, 1965, and the clinical form of the disease, without (type A) and with (type B) evidence of general involvement, was judged by the criteria of the authors (ref. 16).

Clinical aspects in cured malignant lymphogranulomatosis. The patients were grouped according to the Rye stage classification into clinical stages I, II and IV (Table 1). Of the fifteen patients in stage II, fourteen had supradiaphragmatic and only one infradiaphragmatic lymph node involvement. Localized organ lymphogranulomatosis originating per continuitatem from neighbouring lymph nodes was present in the four cured patients in stage IV, the pleura was affected in two, the lungs in two and the thoracic spine in two instances. If these four patients had been classed according to lymph node involvement and the localized organ involvement had been regarded as further lymph node extension, they would belong to spreading stage II (Rye), in contradistinction from the definition of this stage. The Freiburg classification according to MUSSHOFF et coll. (ref. 17, 18) would place all the twenty eight patients in stage I (lymph node in

Table 2

Life expectation in malignant lymphogranulomatosis — Influence of histologic type on survival time according to Lukes & Butler *(1966) — Freiburg patient data 1948—1967*

Histologic type	Numbers	Survival rates in per cent		
		3 year	5 year	10 year
Lymphocytic and histiocytic nodular	11	72.8	46.2	46.2
Lymphocytic and histiocytic diffuse	13	76.9	68.8	58.2
Nodular sclerosis	88	74.5	56.1	36.4
Mixed	40	49.8	36.6	30.5
Diffuse fibrosis	21	43.5	43.5	11.6
Reticular	20	48.1	30.1	30.1
Total	193	63.8	49.0	33.7

volvement on one side of the diaphragm without and with localized organ extension)

The cured patients belong to both clinical forms without as well as with symptoms or signs of active disease the number of patients without signs is only slightly higher than those with signs (Table 1)

Histology in cured malignant lymphogranulomatosis As already stated the original slide preparations from sixteen of the twenty eight cured patients were evaluated and a histologic report was available for a further four patients Of the remaining eight patients either the report or the slide preparation was insufficient or lymph node punctates had been made Twenty patients thus remain for classification according to the histologic criteria of Lukes & Butler (ref 9)

The histologic appearances of the lymphocytic and histiocytic forms consist of a distinct predominance of lymphocytes and histiocytes and only a few Hodgkin and Sternberg giant cells. According to Lukes et coll (ref 10) as well as to the similar Freiburg classification, these forms carry the best prognosis (Table 2)

The diffuse fibrosis and reticular forms in which a marked reduction in lymphocytes and a predominance of Hodgkin and Sternberg giant cells with varying degree of fibrosis occur have a poor prognosis

The prognosis in the nodular sclerosis form is almost as good as in that of the first two groups. All other forms are regarded as mixed forms in which the prognosis is indifferent

Table 3 indicates that most of the twenty classifiable patients considered as potentially cured belonged to the nodular sclerosis group approximately 40 %

Table 1

Distribution of 28 cured patients (13 males and 15 females) according to stage and clinical form of Hodgkin's disease

Clinical form	Stages according to the Rye classification				Number of patients
	I	II	III	IV	
A	6	10	—	—	16
B	3	5	—	4	12
Total	9	15	—	4	28

were evaluated. A classification by the LUKES method was made in four of six patients after the detailed report of histologic investigation, while in a further six patients the diagnosis had been cytologically verified.

Results

The treatment of the 393 patients has been discussed in detail elsewhere (MUSSHOFF & BOUTIS ref. 12, 13, 14). The 5 year survival rate of this series amounted to 48.6 %, the 10 year survival rate to 29.3 %, while the 15 and 20 year survival rates were each 23.1 %.

Spread, signs, histologic types and treatment of the twenty eight cured patients are presented in Tables 1, 3 and 4. The clinical stage was evaluated according to the classification proposed at the Lymphogranulomatosis Symposium at Rye, New York, 1965, and the clinical form of the disease, without (type A) and with (type B) evidence of general involvement, was judged by the criteria of the authors (ref. 16).

Clinical aspects in cured malignant lymphogranulomatosis. The patients were grouped according to the Rye stage classification into clinical stages I, II and IV (Table 1). Of the fifteen patients in stage II, fourteen had supradiaphragmatic and only one infradiaphragmatic lymph node involvement. Localized organ lymphogranulomatosis originating per continuitatem from neighbouring lymph nodes was present in the four cured patients in stage IV, the pleura was affected in two, the lungs in two and the thoracic spine in two instances. If these four patients had been classed according to lymph node involvement and the localized organ involvement had been regarded as further lymph node extension, they would belong to spreading stage II (Rye), in contradistinction from the definition of this stage. The Freiburg classification according to MUSSHOFF et coll. (ref. 17, 18) would place all the twenty eight patients in stage I (lymph node in

Table 2

Life expectation in malignant lymphogranulomatosis — Influence of histologic type on survival time according to LUKES & BUTLER *(1966) — Freiburg patient data 1948—1967*

Histologic type	Numbers	Survival rates in per cent		
		3 year	5 year	10 year
Lymphocytic and histiocytic nodular	11	72 8	46 2	46 2
Lymphocytic and histiocytic diffuse	13	76 9	68 8	58 2
Nodular sclerosis	88	74 5	56 1	36 4
Mixed	40	49 8	36 6	30 5
Diffuse fibrosis	21	43 5	43 5	11 6
Reticular	20	48 1	30 1	30 1
Total	193	63 8	49 0	33 7

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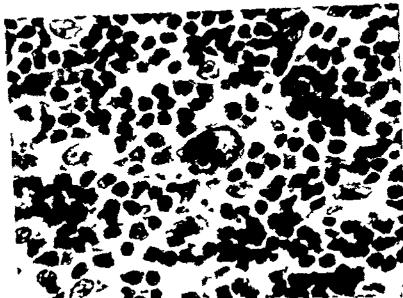


Fig 1 Case 1 (stage I A) L+H diffuse type *Diffuse interspersion* of lymph node by mature lymphocytes Typical Sternberg giant cell with large nucleolus with translucent centre ×900

(FREUDENBERG ref 1), amounted to 22 1 % (the 5 year survival rate of the total series in the period 1948—1967 being 48 6 %) (ref 12 13 14) The concept of cure is thus based upon the fact that the reference curve of patients in the first remission group decreases exponentially and rapidly during the first years after treatment becomes flatter in the years that follow and reaches a constant level at the ninth year of remission (ref 11) No recurrence was recorded in the period 1948—1967 after the ninth year and the statistical probability of a recurrence after 9 years remission is therefore small A patient in whom full remission of 9 years or longer is achieved after the first treatment sequence may be looked upon as cured This premise was taken as a basis for the evaluation of cure used in this investigation

Analysis of the cured patient series as regards clinical aspects histology and treatment produced the following results

1 Cures were achieved only as long as the disease had not crossed the diaphragm whether from above or below Only in one of the twenty-eight patients were the lesions localized infradiaphragmatically (stages I and II, according to Rye) The previously comparatively unfavourable prognosis for infradiaphragmatic locations in clinical stage II and especially stage III (Rye), was due to

Table 3

Distribution according to histologic type (LUKES & BUTLER) *and clinical stage* (Rye) *in cured Hodgkin's disease*

Histologic classification (LUKES & BUTLER)	Numbers	Stage and clinical form							
		I		II		III		IV	
		A	B	A	B	A	B	A	B
Lymphocytic and histiocytic nodular	—	—	—	—	—	—	—	—	—
Lymphocytic and histiocytic diffuse	4	3	—	1	—	—	—	—	—
Nodular Sclerosis	11	1	—	5	3	—	—	—	2
Mixed	4	1	—	2	—	—	—	—	1
Diffuse fibrosis	—	—	—	—	—	—	—	—	—
Reticular	1	1	—	—	—	—	—	—	—
Total patients	20	6	—	8	3	—	—	—	3

(207) of all patients examined in the period 1948—1967 (508) belonged to this nodular sclerosis group. The lymphohistiocytic diffuse form, i.e. the lymphocyte predominat form and the mixed form were present in four of the cured patients. A rare reticular form, previously characterized as Hodgkin sarcoma (JACKSON & PARKER, ref. 3), was present in one patient.

Therapy in cured malignant lymphogranulomatosis. The involved areas were treated with roentgen rays at 180 to 250 kV, adjacent regions were excluded. The radiation dosage in the patient group with lymphocytic or histiocytic proliferation was 2 700 to 3 800 rad, in the group with nodular sclerosis 1 500 to 4 000 rad, in the mixed cell type exposure dose 2 750 R to 4 230 rad, and in the reticular type exposure dose 2 900 R (Table 4). Among the twenty patients classified according to LUKES' scheme, fifteen received chemotherapeutic treatment with nitrogen mustard or cyclophosphamide concurrently or alternatingly. Of the total of twenty eight cured patients, twenty three were given additional chemotherapeutical treatment. The ratio of the patients treated by combined radiation and chemotherapy and those by radiation therapy alone therefore becomes 3 (4 6) : 1 in the group of the 20 (28) cured patients, and 6 1 : 1 in the total of 193 patients treated during the period. None of the cured patients was treated by chemotherapy alone.

Discussion

A certain percentage of patients with Hodgkin's disease can be cured. The percentage during the period 1948—1958, calculated by the life table method

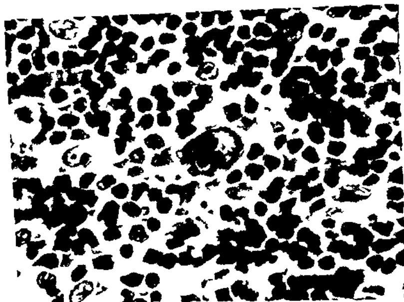


Fig 1 Case 1 (stage I A) L+H diffuse type Diffuse interspersion of lymph node by mature lymphocytes Typical Sternberg giant cell with large nucleolus with translucent centre ×900

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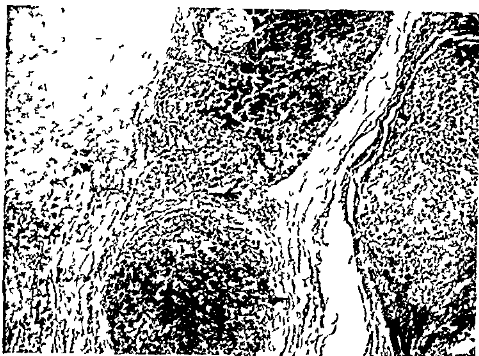
Table 4

Treatment in 20 of 28 cured patients in whom classification according to LUKES & BUTLER *was possible*

Case & sex		Stage	Spread of the disease
Lymphocytic and histiocytic diffuse type (see Fig. 1)			
1	o	I A	Right side of neck
2	♂	I A	Left side of neck and supraclavicular fossa
3	o	I A	Left supraclavicular fossa
10	♀	II A	Left side of neck and supraclavicular fossa Right side of neck and supraclavicular fossa Mediastinum
Nodular sclerosis (see Fig. 2)			
4	♂	I A	Left side of neck and supraclavicular fossa
11	♀	II A	Left side of neck and supraclavicular fossa Mediastinum
12	♀	II A	Left supraclavicular fossa Right axilla and infraclavicular fossa
13	o	II A	Left side of neck Left supraclavicular fossa Mediastinum
14	♀	II A	Right side of neck, supraclavicular fossa and right axilla Left side of neck, supraclavicular fossa and left axilla
15	o	II A	Right supraclavicular fossa Mediastinum
20	o	II B	Both groins
21	♀	II B	Mediastinum Right supraclavicular fossa
22	♀	II B	Right side of neck Right supraclavicular fossa Mediastinum Left supraclavicular fossa Left infraclavicular fossa
25	♀	IV B	Mediastinum, sternum and right lung Left axilla Right axilla *Right supraclavicular fossa* Left supraclavicular fossa
26	o	IV B	Thoracic spine Left side of neck and supraclavicular fossa
Mixed type (see Fig. 3)			
5	♂	I A	Left side of neck, supraclavicular and infraclavicular fossae
16	♀	II A	Right supraclavicular fossa *Left supraclavicular fossa* Mediastinum
17	♀	II A	Left supraclavicular fossa Right supraclavicular fossa Mediastinum
27	♂	IV B	Mediastinum and right paramediastinal pulmonary regions
Reticular type (see Fig. 4)			
[illegible]	[illegible]	I A	Left axilla

Table 4 *(cont.)*

Radiation treatment	Chemotherapy	Remission
3 000 rad/26 days	—	> 13 years
2 800 R/17 days	—	> 13 years
3 000 R/18 days	—	> 11 years
3 500 rad/25 days 2 700 rad/20 days 3 800 rad/26 days	N mustard 44 mg/18 days Cortisone 2 6 g/51 days	> 12 years > 12 years
4 000 rad/26 days	N mustard 26 5 mg/11 days	> 15 years
2 000 R/11 days 1 500 rad/11 days	N mustard 47 mg/20 days Cortisone 1 650 g/33 days	> 13 years
4 000 rad/26 days 3 600 rad/21 days	Sanamycin unknown dose N mustard 31 mg/50 days Decortin 1 32 mg/68 days	> 12 years
3 100 rad/?9 days 3 8?0 rad/37 days 3 750 rad/30 days	N mustard 26 5 mg/34 days Decortin 500 mg/36 days	> 13 years
3 000 R/17 days 3 900 rad/34 days	Cyclophosphamide 7 1 g/39 days Decortin 1 16 g/56 days	> 9 years
3 200 R/23 days 3 300 rad/?3 day	N mustard 36 mg/37 days Decortin 8?0 mg/40 days	> 9 years
2 500 R/23 days	N mustard 41 mg/20 days Sanamycin 5 900 gamma/19 days	> 13 years
3 600 rad/37 days 3 500 R/19 days	N mustard 34 5 mg/20 days Cortisone 5 125 g/61 days	> 12 years
2 8?0 rad 25 days 3 300 rad/41 days 3 200 rad/?5 days 2 800 R/17 days 1 800 R/7 days	Cyclophosphamide 2 9 g/14 days Decortin 875 mg/53 days	> 9 years
4 000 rad/22 days 2 600 R/14 days 2 700 R/19 days 2 800 R/15 days 2 500 R/10 days	N mustard 25 mg/10 days	> 14 years
3 400 rad 20 days 3 000 rad/26 days	Diclo en 39 mg/59 days Decortin 930 mg/64 days	> 9 years
3 000 R/16 days	N mustard 14 mg/6 days	> 14 years
2 7?0 R/33 days 2 500 R/28 days 4 230 rad 31 days	Sanamycin 3 400 gamma/25 days Cortisone 1 650 mg/17 days	> 11 years
2 400 R/28 days ? 400 R/30 days 2 900 rad 25 days	—	> 11 years
3 500 rad 38 days	N mustard 36 mg/20 days	> 19 years
? 900 R/36 days	Decortin 28? mg/22 days	[illegible]



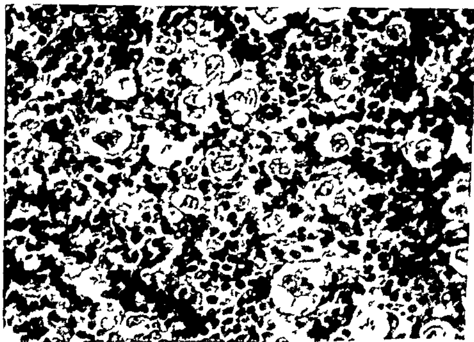


Fig. 2. Case 26 (stage IV B). Nodular sclerosis type. *Upper view*: Knotty broad collagenous fibre bands in centre. v. Gieson. *Lower view*: Lymphocytes, atypical reticular cells and Sternberg giant cells. ×360.

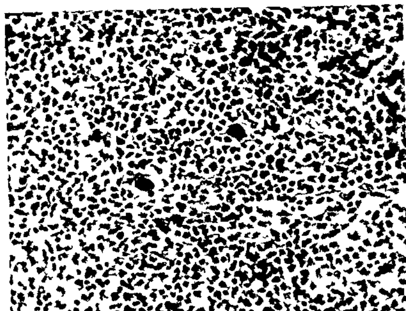


Fig 3 Case 5 (stage I A) Mixed type Varying cell composition with Hodgkin cells Sternberg giant cells histiocytes eosinophil leukocytes plasma cells and slight fibrosis ×360

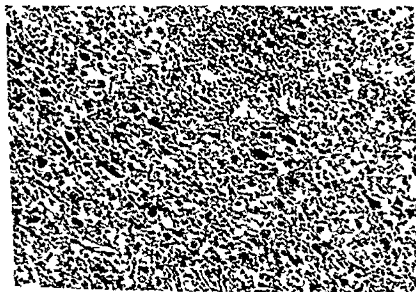


Fig 4 Case 6 (stage I A) Reticular type Predominately atypical reticular cell Sternberg giant cells and Hodgkin cells slight fibrosis and in the germ-

the fact that early diagnosis and thus timely treatment was not possible in the 1940s and 1950s before the introduction of diagnostic lymphography.

One patient in the series has been classed in stage III (Rye) with supra- and infradiaphragmatic lymph node involvement and is in the eighth year of his first remission, he therefore almost fulfills the statistical qualifications for complete healing. VERA PETERS and KAPLAN (personal communications) have also had several cured patients in stage III.

Systematic irradiation of all supra- and infradiaphragmatic lymph node sites in stage III with telecobalt was used from 1964 (ref. 27) for twenty of the twenty-five patients in stage III. (One patient died before infradiaphragmatic irradiation could be commenced after the supradiaphragmatic irradiation, and two patients discharged themselves before termination of therapy. Endolymphatic therapy took the place of infradiaphragmatic percutaneous irradiation in two patients.) The remission rate in the series treated in this way thus amounted to 81 %. It would therefore appear that patients with both supra- and infradiaphragmatic lymph node involvement stage III (Rye) may now be curable.

2. The presence of clinical symptoms and signs of the disease (clinical form B) *does not exclude cure*. The ratio of cured patients with active manifestations to those without in the present series is 4 to 3. This is contrary to the widely held opinion that manifestations according to clinical form B should be regarded as a contraindication to further local treatment.

3. Sex has no important influence on cure. In spite of the larger number of males in the series, the females outnumbered the males by about 3 : 2.

4. Cure is evidently possible in all histologic forms. This is true for the lymphocytic and histiocytic predominant groups and the lymphocyte depleted forms, as well as for nodular sclerosis and mixed forms. By far the largest number of cured patients (three out of four) belong, however, to the prognostically favourable lymphocyte predominant forms and the nodular sclerosis group, as was to be expected. The approximate ratio of possible cure when compared in the four histologic groups, i.e. the lymphocytic predominance, the nodular sclerosis, the mixed type and the lymphocytic depletion groups is 7 : 5 : 4 : 1 in the whole material.

5. All cured patients were treated by local radiation therapy (180 to 250 kV). Some of the cured patients received additional chemotherapy. One patient with reticular fibrosis in stage I was given combined surgical and radiation treatment. None of the cured patients was treated by chemotherapy alone. Present-day therapeutic possibilities suggest that complete cure is attainable — if the few cases in the literature treated purely by surgery are disregarded (SLAUGHTER ref. 25, 26) — by irradiation alone.

The doses given to cured patients varied from 1 500 to 4 000 rad. This points

to the fact that the radiation dose to be employed for destroying the malignant tissue in lymphogranulomatosis cannot be fixed as it must differ from patient to patient (PAPILLON et coll (ref 21) made the same observation) The level of the tissue destructive dose, empirically determined previously to 4 000 R focal dose (ref 4 19), is the one which is tissue destructive for practically all (more than 95 %) Hodgkin granulomas As long as the specific tissue destructive dose for each patient with lymphoma malignum Hodgkin is not calculable in advance the empirically determined destructive dose of 4 000 rad should for security reasons always be given

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SUMMARY

Of a series of 393 patients with Hodgkin s disease treated during the period 1948—1967 22 1 % was cured Twenty seven of the patients had supradiaphragmatic and only one infradiaphragmatic lymph node involvement in stages I and II (Rye) while four had extension to a local organ (stage IV) Almost the same number of males and females were affected All patients were irradiated and some received additional chemotherapy lymph node extirpation was performed in one patient

ZUSAMMENFASSUNG

Erfolgreiche Heilung konnte in 22 1 Prozent von 393 Patienten mit Hodgkinscher Erkrankung die *in den Jahren 1948—1967* behandelt wurden festgestellt werden Siebenundzwanzig Patienten hatten Lymphdrusenbefall oberhalb des Zwerchfelles und nur ein Patient unterhalb des *Zwerchfelles* im Stadium I und II (nach Rye) und vier Patienten hatten Befall eines benachbarten Organes (Stadium IV) Weibliche und mannliche Patienten *waren ziemlich* gleichmassig betroffen Samtliche Patienten erhielten Strahlenbehandlung *und einige* erhielten ebenfalls Chemotherapie Exstirpation von Lymphknoten wurde in einem Patienten vorgenommen

RÉSUMÉ

Sur 393 *malades atteints* de maladie de Hodgkin traites au cours de la periode 1948—1967 un total de 22 1 pour cent ont ete gueris Vingt sept de ces malades avaient une atteinte ganglionnaire *sus diaphragmatique* et un seul malade une atteinte ganglionnaire sous-diaphragmatique au stade I et II (Rye) et quatre malades avaient une extension localisee à un organe (*stade IV*) *Les* hommes et les femmes etaient atteints presque dans les memes proportions Tous les malades ont ete irradies et certains ont subi en plus une chimiotherapie L'extirpation des *ganglions* a été faite chez un malade

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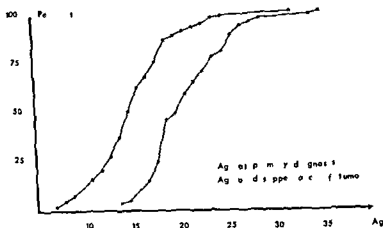
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Age at the primary diagnosis in 69 patients and age on disappearance of the tumour in 63 patients with nasopharyngeal angiofibroma illustrated by cumulative distribution curves

varied from 7 to 32 years 80 % being between 10 and 21, with a mean age of 15 years (see accompanying diagram)

Symptoms Nasal obstruction was the most common symptom and occurred in sixty three patients (91 %), combined in forty one patients (59 %) with spontaneous bleeding, which was often profuse and necessitated medical attention The patients seen before they were 14 years of age usually had a short history and in fourteen out of nineteen it did not exceed one year Half the number of those examined after the age of 16 had a history of more than a year and the patient admitted for treatment at the age of 32 had his first symptoms 5 years previously

Gradual expansion of the tumour was evident in fourteen patients from bulging of the cheek or exophthalmus In one instance intracranial spread of the tumour was found post mortem In fifty three patients the tumour was confined to the nasopharyngeal space sometimes with extension into the nasal cavities or the paranasal sinuses or both, as shown below in a total number of 68 patients (one case was unclassified) the tabulation giving the location and spread of the tumour

Nasopharyngeal and posterior region of the nasal cavities	29	(43 %)
Nasopharyngeal space nasal cavities and the paranasal sinuses or both	24	(35 %)
Nasopharyngeal space the orbital cavity the infratemporal fossa or both	14	(12 %)
Nasopharyngeal space and intracranial extension	1	(1 %)

FROM RADIUMHEMMET, THE DEPARTMENT OF OTORHINOLARYNGOLOGY, AND THE DEPARTMENT OF CLINICAL RADIOPHYSICS OF THE NATIONAL INSTITUTE OF RADIATION PROTECTION, KAROLINSKA SJUKHUSET, STOCKHOLM, SWEDEN

JUVENILE NASOPHARYNGEAL ANGIOFIBROMA

A clinical study of 69 cases

by

BERTA JEREB, A. ÄNGGARD and I. BARYD

Juvenile nasopharyngeal angiofibroma was defined by MARTIN et coll. in 1948 as follows: '... a specific highly vascular, non infiltrating essentially benign neoplasm, occurring in the nasopharynx or posterior nasal cavity of pubescent males. Symptomatically the tumour is characterized by nasal obstruction, repeated epistaxis and by progressive growth throughout the period of adolescence, with a tendency towards spontaneous regression at about the time of sexual maturity.' This description is still in line with current concepts of this uncommon growth but it is evident from the numerous articles relating to its etiology (14, 15), behaviour and treatment (*1—10, 12—16*) that some uncertainty *still* exists.

Material. Our investigation was based on an analysis of 69 patients admitted to Radiumhemmet during the period 1919—1966 with a diagnosis of nasopharyngeal angiofibroma. In sixty one of these, the diagnosis was confirmed by histologic examination. All the patients were males, age at primary diagnosis

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operation had previously been performed and all were referred for radiotherapy Fifty patients had only radiotherapy (see Table 1)

Percutaneous roentgen treatment was, until about 1947, usually combined with the local application of a radium tube, or an interstitial needle implant in large bleeding tumours A 5 g short distance radium unit was used in four patients a ^{60}Co unit in three and the 16 MV roentgen beam from a betatron in one patient Irradiation with the ^{60}Co unit and the betatron was administered through two opposite portals while up to five portals were used with conventional 170 to 200 kV radiotherapy The eyes were always protected The treatment was sometimes repeated up to five times over a 2 to 3 year period

A retrospective calculation of the tumour dose (in rad) in the nasopharynx has been attempted The figures are, of course only approximate especially where radium was applied locally The relationship between the tumour dose and the type of therapy is presented in Table 1 In 74 % of the patients receiving conventional roentgen irradiation the tumour dose in the nasopharynx was less than 3 000 rad When roentgen irradiation was combined with local radium therapy a higher dose was usually obtained, and in 57 % of the patients it exceeded 3 000 rad

High irradiation doses were applied until 1957 Between 1919 and 1926 the dose to the nasopharynx exceeded 6 000 rad in six of nine patients and the tumour dose was more than 10 000 rad in three patients when radium was applied locally in combination with external irradiation A tendency has arisen since 1957 to use lower doses (Table 2)

Results

Three of the sixty nine patients were lost to follow up since the last treatment they had been free from the condition Two patients treated during the previous three years had small residual growths one and two years after admission respectively One patient who was alive 16 years after the first treatment has not been seen since treatment

Of the remaining sixty three patients, forty seven have been free from the disease for more than 10 years ten for more than 5 years and six for more than one year as may be seen from the following tabulation

Follow up pe iod after recovery (years)	1	2	5	10	20	30	>40
Number of pat nts	2	4	10	18	13	14	2

Five patients died of intercurrent disease after 11 years with no signs of a growth The mean time elapsing from the beginning of therapy to the disappearance of the tumour was 5 years the average age being 20 years As many as

Table 1

Methods of treatment in a total of 69 patients and tumour dose when radiotherapy was given

Tumour dose rad	Operation alone	Operation plus roentgen therapy	Operation plus roentgen therapy plus Ra*	Roentgen therapy alone	Roentgen therapy plus Ra	Roentgen therapy plus operation	Total
No radiotherapy	3						3
<2 000		3	1	9		1	14
2 000—3 000		1	1	20	1	1	24
3 000—6 000		3	3	8	6		20
>6 000			2	1	5		8
Total	3	7	7	38	12	2	69

* Local application of radium

Table 2

Doses given at different periods in the treatment of a series of 66 patients

Treatment period in relation to present time	Number of patients receiving doses (in rad)				Total number of patients
	<2 000	2 000—3 000	3 000—6 000	>6 000	
More than 10 years ago	1	4	1	—	6
10—20 years ago	3	6	1	1	11
20—30	3	6	8	1	18
30—40	6	7	9	—	22
More than 40 years ago	1	1	1	6	9
Total	14	24	20	8	66

Methods of treatment Over the period 1919—1966 the attitude to juvenile angiofibroma has undergone a change The clinical appearance of the tumour prompted attempts at therapy and all the patients admitted in the early decades received treatment With the better understanding of the benign nature of the growth in recent years, however, a more cautious approach has been adopted, and this since 1957 Only five patients have been treated at Radiumhemmet in the interval, and after 1966 none at all

Operation, as the sole form of treatment, was performed in three patients with small tumours, one of these required a further operation Two patients were given radiotherapy prior to surgery In fourteen patients, at least one

Table 3

Radiation dama e occurrin, in relation to the treatment doses given

	<2 000 rad	2 000—3 000 rad	3 000—6 000 rad	>6 000 rad
Atrophic changes in sk n or nasal mucosa	6	10	8	7
Cataract		1	1	
Osteonecros s				1
Total	6/14 (42)	11/24 (45)	9/20 (45 %)	8/8 (100 %)

cavity and caused bulging of the right cheek and palate and slight exophthalmus Biopsy confirmed the clinical diagnosis of nasopharyngeal angiofibroma

Conventional roentgen treatment was instituted through four facial portals with an ap proximate dose of 1 000 rad to the nasopharynx for 2 weeks Two months later enlargement of the tumour was noticed together with impairment of the vision of the right eye through atrophy of the optic nerve

As treatment almost identical to the first one was without effect operation through a right Denker approach was performed The posterior and medial walls of the maxillary sinus were bulging forwards and laterally but there was no destruction of bone there was expansive growth through the medial wall of the orbit into the ethmoid and sphenoid re gions. The neoplasm was removed piecemeal with moderate bleeding but the patient died under the operation Autopsy disclosed destruction in the ethmoid and sphenoid regions with *extension of the tumour (1 cm × 1 cm)* into the middle cranial fossa There was no invasion of the dura The diagnosis of nasopharyngeal angiofibroma was confirmed histologically

Case 2 A 14 year old boy was referred to Radiumhemmet in 1923 with nasal obstruction and epistaxis for 6 months A tumour growing from the nasopharynx into the left nasal cavity and causing bulging of the cheek and exopthalmus was diagnosed as a juvenile *nasopharyngeal angiofibroma*

Conventional roentgen treatment with a tumour dose of 1 300 rad to the pharynx was given for 6 weeks through four facial portals but with no noticeable improvement Three months later a further 1 800 rad was administered by the same *technique* and in addition 900 rad was given to the nasopharynx with a 5 g radium unit through the same facial fields Local radium was also applied on several occasions about 2 000 rad intracavitarily to the nasopharynx and 1 700 rad as a needle implant The last treatment was administered in 1926 when the tumour was large and there was bulging of the cheek and exophthalmus The total dose to the nasopharyngeal region was calculated to be 7 900 rad

The patient remained under observation and the tumour gradually diminished until 1933 when it had disappeared Atrophic changes of the nasopharyngeal and nasal mucosa together with impairment of the vision of the left eye due to cataract of the lens had how ever developed. The patient s condition remained unchanged until 1958 when he developed left dacrocystitis Conservative treatment and antibiotics were without effect *Sequestrated bone fragments* of the maxilla were removed at two op rations but no improvement was achieved The patient died in 1960 at the age of 51 from frontal osteitis and meningitis

80 % of all the patients were free from the disease before they were 25 years old The condition lasted longer in patients under 14 years of age than in those who were older, more treatments were then administered although the total tumour dose was usually lower A higher tumour dose and a longer duration of the disease was the rule in patients with tumours spreading to the infratemporal fossa

Of the fifty eight patients in whom the time between primary therapy and regression of the tumour could be established, forty nine had objective as well as subjective improvement within 10 months, when a tumour dose of about 2 000 rad had been given for 4 weeks In the remaining patients, the period of regression was as long as one or two years The tendency to bleeding usually diminished during radiotherapy

Radiation damage in relation to the follow up time after the first treatment occurred as follows

Follow up period (years)	10	11—20	21—30	31—40	>40
Patients with radiation damage	0/6 (0 %)	3/12 (25 %)	7/16 (43 %)	14/22 (63 %)	7/7 (100 %)

Radiation damage occurring in relation to the treatment doses given is recorded in Table 3 Atrophic changes of the nasal and nasopharyngeal mucosa, such as dryness, formation of crusts and chronic rhinitis, were present in thirty one patients, in two of whom the changes were quite severe, with a tendency towards recurrent spontaneous epistaxis They were seen in sixteen of the patients (40 %) given less than 3 000 rad The proportion of patients with such changes increased with the time after treatment and they occurred in all the seven patients followed for more than 40 years, though two of them had radiation doses to the nasopharynx of between 2 000 and 3 000 rad All the patients in whom the tumour dose was at least 6 000 rad and those who received local radium treatment had signs of irradiation damage Two patients undergoing radiotherapy developed cataract and one patient osteonecrosis Malignant degeneration was not encountered

Two patients died from complications in the treatment It would seem to be of interest to report briefly on these

Case reports

Case 1 This was a 7 year old boy admitted to Radiumhemmet in August 1946 who had had symptoms of progressive nasal obstruction for the two previous months The nasopharyngeal space was occluded by a lobulated tumour which extended into the right nasal

disease and the low age of the patients radiotherapy is inadvisable When treatment is necessary because of bleeding nasal obstruction or excessive growth, a surgical procedure, though not necessarily a radical one is to be preferred Preoperative radiotherapy may be used in an attempt to reduce bleeding or the operative risk

Cases of nasopharyngeal angiofibroma have been reported where no complications have followed the use of 6 000 rad to the nasopharynx (7) On the other hand, doses of 1 000 to 2 000 rad have been deemed sufficient (1, 12) and complications after 3 000 rad have been negligible (7) In our experience the tumour dose need not exceed 3 000 rad to be given over about 6 weeks

SUMMARY

A series of 69 patients with juvenile nasopharyngeal angiofibroma admitted during 1919—1966 has been analysed Surgical measures were taken in three of the patients and sixty six were given radiotherapy The clinical features treatment and results are discussed The treatment of nasopharyngeal angiofibroma should be directed against manifestations such as bleeding nasal obstruction and excessive growth Surgery is the method of choice

ZUSAMMENFASSUNG

Eine Serie von 69 Patienten mit nasopharyngealem Angiofibrom die zwischen 1919 und 1966 behandelt wurde wurde kritisch analysiert Operation erfolgte in drei und Bestrahlung in sechsundsechzig Patienten Das klinische Bild die Behandlung und die Resultate werden diskutiert Die Behandlung des nasopharyngealen Angiofibroms sollte nur symptomatisch sein um die Blutung die Nasenverstopfung und zu grosse Ausbreitung des Tumors zu verhindern Die Operation ist die Methode der Wahl

RÉSUMÉ

Les auteurs ont analyse une serie de 69 malades atteints d angio fibrome naso-pharyngien du jeune hospitalises entre 1919 et 1963 Trois de ces malades ont ete traites chirurgicalement et soixante six par radiothérapie Les auteurs etudient les caracteres cliniques le traitement et les resultats Le traitement de l angio-fibrome naso pharyngien devrait etre dirigé contre l hemorragie l obstruction nasale et le volume tumoral excessif La chirurgie est la méthode de choix

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Discussion

Isolated cases of nasopharyngeal angiofibroma have been reported in females (5, 7, 8, 13, 14, 17) and at advanced ages (5, 8, 17), but the correctness of the diagnosis in many of these patients has been challenged (1, 10, 12) As all our patients were males and were seen before the age of 30, and most of the patients reported in the literature have been young males, a diagnosis of this condition in a female, or at middle age or over, is open to question

An inverse relationship between the growth of a nasopharyngeal angiofibroma and age has frequently been noticed (1, 5, 10, 12, 13, 14) In the present series the duration of the disease was longer where the tumour appeared before puberty Furthermore, the tumour seemed to be more active with a short duration of symptoms These findings suggest that the growth of the nasopharyngeal angiofibroma is stimulated by hormonal factors before and during puberty, and that once sexual maturity is reached this effect is abolished

Indications, methods, hazards of radiation, surgical therapy and a combination treatment have been dealt with by various authors (5, 6, 8, 9, 10, 12, 19) All the methods used have proved successful and appear to have yielded practically equivalent results Regression after radiation is slow to begin (7, 10) and the duration of the disease is usually longer (1, 10) In the series in which surgical treatment has been the chief approach (10) an 80 % cure rate has been reported under the age of 20, in the present series this figure was not recorded in patients under the age of 25

The frequency of irradiation complications increased with the time elapsing after treatment though the dose was higher for the patients treated before 1927 (see Table 3) Younger patients with a long life expectancy are more likely to contract irradiation damage The risk of inducing a malignant tumour must also be taken into consideration

Both radiotherapy and surgery have been causes of fatal outcome, usually through aggressive attempts at complete irradiation of the tumour (1, 5, 6 8, 10, 12) Two of the patients in our series died from complications of the treatment, one after extensive surgery and the other after irradiation In the former, the intracranial spread of the tumour led to primary optic atrophy, this has also been reported by other workers (2, 3, 13) As patients with intracranial spread are considered to be poor surgical risks (2, 5) polytomography and angiography should be performed preoperatively when optic atrophy is present

The treatment of nasopharyngeal angiofibroma seems to be more hazardous than the condition itself and should be given only after careful deliberation, and then be focussed on the symptoms In view of the benign nature of the

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EVALUATION OF RADIATION TREATMENT OF PAINFUL CONDITIONS OF THE LOCOMOTOR SYSTEM

A double blind study

by

IAN GOLDIE BENGT ROSENGREN ERIK MOBERG and ERLAND HEDELIN

Inflammatory and degenerative changes in joints and adjacent structures often cause pain the exact mechanism by which this is elicited is not completely understood (BONICA 1953) It has been suggested that oedema following the increased capillary permeability in inflammation may exert pressure on nerve endings and result in pain (LINDAHL & REXED 1950 OLSSON 1958 ASBOE HANSEN 1963 HEIMANN 1964 CABITZA 1965 SICUTERI 1965, BALLABIO 1965) Impairment of venous drainage from subchondral bone as part of the inflammatory reaction may also contribute to pain (HULTH 1969) The alleviation of pain that takes place after the administration of antiphlogistic drugs would to some extent support these opinions

A relationship may exist between increased hydrogen ion concentration and pain — as evident in inflammatory conditions (MENKIN 1937 ROPES et coll 1953 CUMMINGS et coll 1966 GOLDIE et coll 1969) REVICI et coll (1949) believed that local changes in damaged tissues may bring about a lowering of the nerve threshold for pain and that end organs ordinarily concerned with other

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Table 1

Distribution accordin to age and sex in the two groups subjected and not subjected to radiation

	Group given radiation treatment			*Group not receiving treatment*		
	Men	Women	Total	Men	Women	Total
20—30	4	1	5	3	1	4
31—40	4	7	11	9	6	15
41—50	22	19	41	18	19	37
51—60	27	35	62	19	29	48
61—70	20	28	48	28	29	57
71—80	10	20	30	11	16	27
81—90	3	5	8	1	5	6
			205			194

having carried out no systematic investigation on the results of radiation treatment in joint disorders were nevertheless of the opinion that it may be of benefit LEQUESNE (1967) found that radiotherapy had no effect in osteo-arthritis of the hip joint except when there was an associated trochanteric bursitis

Evaluation of the results in a special treatment of inflammatory and degenerative joint disorders becomes difficult as the course is most variable Moreover also the radiation treatment varies both with regard to fractionation and total dose Some believe in smaller doses at repeated intervals (OCHSNER et coll 1940 STOLL 1957 HULTBERG et coll 1963) e g 25 to 100 R a day for 3 to 5 days Others find an initial treatment with up to 400 R daily with a total of 2 400 R in a week to be of greater benefit (DE LORIMIER 1937) and HORWITZ et coll (1944) suggested 250 R a week for 6 or 7 weeks

Difficulties in the evaluation of results of a therapeutic agent may thus arise when the disease for which a patient is treated causes symptoms that at least for some time subside spontaneously and also when the mode by which the therapeutic agent is applied varies considerably Controlled series have as yet not been encountered excepting one reported as a discussion by NEWELL in 1939 and one by PLENK in 1952 In the former series boils in alternate cases were shielded from the radiation by a lead filter practically no difference in the clinical course of the whole eries was apparent PLENK reported no difference in the results of a treated and non treated controlled series of calcifying tendinitis of the shoulder LINDAHL & BACKLUND (personal communication) in a double blind study recorded no difference in the results of treated and untreated groups with joint disorders

The aim of the present study was to compare two groups of patients with

forms of sensations are altered in a way to cause the impulses originated to evoke the sensation of pain

LINDAHL (1961) has demonstrated the relationship between increased hydrogen ion concentration and severe pain in the skin, whereby the hydrogen ion may be the chemical mediator that triggers the pain stimulus in the nerve endings Alkalinization alleviated pain

Painful conditions of the locomotor system — possibly due to the above mechanism — have long been regarded suitable for radiation treatment Proliferating tissue, such as endothelium in newly formed blood vessels in the subchondral region, and partly the region of the inflammatory reaction in the joint capsule at its junction with the bone cartilage, are sensitive to ionizing radiation On the other hand, PENDERGRASS et coll (1941) and ELLINGER (1957) stressed *that irradiation transfers an area of passive hyperemia into active hyperemia* which among other things decreases oedema and increases in lymphatic flow White blood cells and particularly the lymphocytes are still more radiosensitive and react to low doses of one or a few hundred rad (TROWELL 1952)

Irradiation may cause slight initial acidosis which however is followed by a longstanding alcalosis (ELLINGER 1957) Another factor that is also influenced by irradiation is the calcium content of tissue This is increased, which leads to a *more marked anti oedematous effect (ELLINGER 1957, HULTBERG et coll 1963)*

Radiation treatment is supposed to counteract the establishment of certain strongholds for the maintenance of the inflammatory reaction that leads to pain in some common joint and juxta articular disorders Suitable doses for treatment of these diseases are considered to be in the region of a few hundred R and not over 1 000 R in one series A total dose of up to about 2 000 R is considered to produce no disturbing late side effects, such as fibrosis and impairment of the *mobility of the joint*

In the treatment of spondylosis deformans of the spine the doses have to be low due to the risk of neoplastic bone marrow disease (GRAHAM 1960)

The conception that pain relief can be attained has been widely accepted but little reported SANDSTROM et coll (1937) gave details of successful treatment OCHSNER et coll (1940) published the results in fifty two patients with post traumatic pain There was relief of pain in thirteen out of twenty seven post fracture patients, in six out of fourteen posttraumatic patients without fracture, and in five out of eleven patients with joint pain following operation Relief was therefore obtained in approximately 50 % HORWITZ et coll (1944) reported successful treatment in nine patients with hydroarthrosis of the knee joint STOLL (1957) assessed the relief of pain in sixty two *patients receiving radiation therapy* for posttraumatic arthrosis, para arthrosis and fasciitis Alleviation was reached in 50 % of patients within 1 to 4 weeks HULTBERG et coll (1963), though

treated locomotor ailments amounting to 439 are given in Table 2 Roentgen examinations were performed in all the patients prior to the treatment in order to exclude malignant or other disease not suitable for this type of treatment

All the patients were before the treatment individually interrogated and examined by an orthopaedic surgeon who did not know which patients were to receive treatment The diagnosis was modified when it did not agree with the initial one A radiologist selected the area to be treated The patients in one of the groups were subjected on alternate days to a series of roentgen treatments according to a given procedure Every second day this procedure was repeated for the other group of patients but then irradiation was prevented by a lead diaphragm The procedure was repeated for both groups until conclusion of the treatment period Neither the patient, nor the orthopaedic surgeon the radiologist or the referring physician were to know who had received the true radiation treatment

Six weeks following the conclusion of the treatment procedures control of the patients was made by the orthopaedic surgeon who still did not know which patients had received irradiation The interrogation was thus renewed and included post therapy results and an objective examination The fee for treatment as decided by the National Health Insurance representative was paid by all patients irrespective of treatment or not When the follow up study was terminated the code for the treated and untreated patients was released and studied by the orthopaedic surgeon and the radiologist

The following factors were applied in the radiation treatments 170 kV HVL 1 mm Cu and usually 40 cm SSD

The shoulders were irradiated with 10 cm × 12 cm collimators Two opposing beams were used with an exposure of 3 × 150 R In acute cases the exposures were lower i e 75 or 100 R Knees were also irradiated with two opposing fields with the same collimators and 3 × 200 R Hips were treated with three treatment fields one ventral one lateral and one dorsal the field size being 9 cm × 15 cm The SSD in these treatments was 50 cm and the exposure per field 3 × 200 R Trochanteritis was treated with one ventral and one lateral field applying the same field size and dose

Patients with spondylosis deformans cervicalis thoracalis et lumbalis were treated with two dorsal fields angled at 25 to 30 In the cervical spine the collimators measured 4 5 cm × 12 cm and in the thoracic and lumbar spine the fields were generally 6 cm wide and 10 to 15 cm long The SSD for the cervical spine was 40 cm and for the thoracic and lumbar spine 50 cm Every field was exposed to 3 × 200 R Epicondylitis was treated with a field measuring 6 cm × 8 cm and 4 × 150 R Patients with calcaneodynia were treated with the same tubes and two opposing fields one medial and one lateral the exposure being 3 × 150 R

Table 2

Diagnoses and number of patients in the two groups which received respectively true and 'false' treatment for different disorders

	Radiation treatment	False radiation treatment	Total
Spondylosis			
Cervical	34	47	81
Thoracic	—	1	1
Lumbar	2	4	6
Arthrosis (osteoarthritis)			
Arthrosis def art hum scap (roentgen evidence of cartilage destruction of hum head)	3	7	10
Arthrosis def art acromioclav	3	6	9
Arthrosis def art cubiti	—	1	1
Arthrosis def art carpometacarp	5	1	6
Arthrosis def coxae	14	9	23
Arthrosis def genus	53	39	92
Arthrosis def art talocrur	2	2	4
Arthrosis def art metatarsophal	3	—	3
Tendinitis			
Peritendinitis hum scap	70	71	141 (5 acute cases)
Epichondylitis lat et med hum	11	14	25
Trochanteritis	4	2	6
Tendinitis lig coll regio genus	1	—	1
Calcaneodynia (spur and plantar bursitis)	5	4	9
Synovitis			
Genus	2	—	2
Metatarsophal	1	—	1
Other	10	8	18
			Total 439

ailments of the locomotor system, only one of which received radiation treatment using a double blind technique

Material and Methods

The series consists of 399 patients referred for disorders of the locomotor system The age and sex distribution appear in Table 1 and the diagnoses of the

Table 4

Diagnoses and subjective and objective evaluations of the results in the group not exposed to radiation

	Total	Subjective evaluation		Objective evaluation		
		Improvement	Same or worse	Improvement	Same	Worse
Spondylosis cervicalis cum rhizopathia	47	33	14	20	20	3
Spondylosis thoracalis						
Spondylosis lumbalis	5	1	4	1	4	—
Periarthritis hum scap	71	47	24	38	19	7
Epicondylitis lat et med hum	14	9	5	8	4	1
Arthrosis def art hum scap						
Arthrosis def acromioclav	13	10	3	9	2	—
Arthrosis def art cubiti	1	1	—	1	—	—
Arthrosis def art carpomet carp	1	1	—	1	—	—
Trochanteritis	2	1	1	—	1	—
Calcaneodynia	4	3	1	3	1	—
Tendinitis reg o genus						
Synovitis genus	—	—	—	—	—	—
Synovitis metatarsophal	—	—	—	—	—	—
Arthrosis def coxae	9	5	4	1	6	—
Arthrosis def genu	39	23	16	9	28	2
Arthrosis def art talocrur						
Arthrosis def art met tarsophal	2	1	1	1	1	—
Other	8	4	4	1	6	1
Total	216	139 (64 %)	77 (36 %)	93 (43 %)	92 (43 %)	14 (6 %)

During all the treatments (true or false), a dental *roentgen film* was placed in the beam as a check. It was of course blackened in all *the true treatments* and was unexposed in the false ones.

All the patients were directed not to take any antiphlogistic or other drugs nor physiotherapy for their ailments during the treatment period or during the subsequent follow-up period.

Results and Discussion

A total of 395 out of the 399 patients were controlled. One patient had died and three could not be traced. The overall results with analyses of the diagnoses

Table 3

Diagnoses and subjective and objective evaluations of the results in the group treated with radiation

	Total	Subjective evaluation		Objective evaluation		
		Improvement	Same or worse	Improvement	Same	Worse
Spondylosis cervicalis cum rhizopathia	34	21	13	14	18	2
Spondylosis thoracalis						
Spondylosis lumbalis	2	1	1	1	1	—
Peritendinitis hum scap	70	52	18	31	3[illegible]	4
Epicondylitis lat et med hum	11	5	6	4	6	1
Arthrosis def art hum scap						
Arthrosis def acromioclav	6	5	1	3	3	—
Arthrosis def art cubiti	—	—	—	—	—	—
Arthrosis def art carpometacarp	5	4	1	2	3	—
Trochanteritis	4	3	1	2	1	1
Calcaneodynia	5	4	1	3	2	—
Tendinitis regio genus						
Synovitis genus	3	3	—	2	—	—
Synovitis metatarsophal	1	—	1	—	1	—
Arthrosis def coxae	14	6	8	4	7	2
Arthrosis def genus	53	37	16	22	30	1
Arthrosis def art talocrur						
Arthrosis def art metatarsophal	5	4	1	2	2	1
Other	10	7	3	5	1	—
Total	223	152 (68 %)	71 (32 %)	95 (43 %)	107 (48)	12 (5 %)

No collimators were used in other regions The areas around the treatment fields were protected with lead rubber

Osteoarthrosis of the joints of the hand was irradiated with 3 × 150 R to one volar and one dorsal field Osteoarthrosis of the joints of the foot was treated in the same way with two opposing fields and 3 × 200 R Osteoarthrosis of the metatarsophalangeal joints was treated with one dorsal field and 4 × 150 R

The depth doses calculated in the different treatment regions were approximately between 500 and 1 000 rad

4) may be noted, however In Table 3 the subjective improvement is 68 % and the objective improvement 43 % In Table 4, the subjective improvement is 64 % and the objective improvement 43 % The difference between the subjective evaluations in the two groups may to some extent depend on the failure of some patients to come to the interrogation and on the difficulty of obtaining a satisfactory after history However the recorded evaluations of subjective improvements are about the same for both groups of patients, exposed or not exposed to the radiation treatment and there is no difference between the two groups in the evaluations of objective improvements

Our observations are further substantiated by the general individual opinions about the treatments A strict correlation could be established between satisfied and subjective improvement as mentioned in Tables 3 and 4 In the group exposed to radiation 66 % satisfied correspond to 68 % subjective improvements in Table 3 In the group not exposed to radiation 63 % satisfied correspond to 64 % subjective improvements in Table 4 The results obtained correspond well with what is generally accepted by radiotherapists HOWARD (1957) reported that 70 % of the patients with spondylarthrosis improved after radiation treatment

A question commonly discussed is the time from final treatment to improvement In this study improvement appeared to occur after 2 to 3 weeks both in the exposed and the unexposed groups (Table 5)

The tendency in the whole series as such was thus improvement after a few weeks whether radiation treatment was given or not Further analysis of the three largest groups humeroscapular peritendinitis osteoarthrosis of the knee and cervical spondylosis revealed that they together comprised 75 % of the total material or 157 patients in each of the exposed and unexposed groups As for the subjective estimation of improvement in the exposed group it was observed in 74 % of humeroscapular peritendinitis in 70 % of osteoarthrosis of the knee and in 62 % of cervical spondylosis With 68 % improvement in the total exposed group there is thus good correlation between the subgroups and the whole of the exposed group

Corresponding observations were made as regards the objective evaluation 44 % of humeroscapular peritendinitis 42 % of osteoarthrosis of the knee and 41 % of cervical spondylosis or 43 % of the total exposed group were improved

As for the group not exposed to radiation treatment similar trends in the subjective estimation were registered The improvement for humeroscapular peritendinitis was 66 % for osteoarthrosis of the knee 59 % and for cervical spondylosis 70 % or 64 % in the total series

The objective evaluation revealed better results in humeroscapular peritendinitis where improvement occurred in 52 %, as compared to 43 % of the total un

Table 5

Time for improvement following termination of treatment

	During treatment	Immediately after treatment	Weeks after treatment				
			1	2	3	4	5
Exposed to radiation	5	7	29	29	47	15	7
Not exposed to radiation	10	7	16	28	35	13	8

Table 6

Increased pain or other local reaction during treatment in relation to result of irradiation

	Pain during treatment	Subjective result after treatment	
		Improvement	Worse
Exposed to radiation	12	7	5
Not exposed to radiation	11	6	5

The discrepancy in the respective numbers of patients with subjective and objective evaluations is due to the failure of some patients to come to interrogation The treatment, according to the patients' opinions, was satisfactory in 148 of those who had received radiation treatment and in 135 of those who had not been exposed to radiation, whereas dissatisfaction was recorded for 75 of the patients who had received radiation treatment and 81 of those who had not been exposed to radiation

The time for improvement following the termination of the series of treatments is recorded in Table 5 A number of patients had some difficulty in giving accurate information and others stated that the pain had subsided gradually Pain and local discomfort during the treatment period were also registered and related to the end results with a view to find out if these could have been influenced by the reactions (Table 6)

No true differences in the follow up results, with the doses used in this investigation, could be established between the group exposed and the group not exposed to radiation treatment

Certain differences in the subjective and objective registrations (Tables 3 and

RÉSUMÉ

Les auteurs ont étudié une série de 399 malades correctement controles et representant 439 atteintes douloureuses de l appareil locomoteur Ils sont arrives à la conclusion que le resultat final est le meme chez les malades traités par radiothérapie pour ces affections et chez les malades qui n ont pas subi ce traitement

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exposed group Osteoarthrosis of the knee was somewhat worse with only 23 % improvement, but cervical spondylosis with an improvement of 42 % corresponded well to the figure for the total group It has not been possible to find any obvious reason for this

There was no statistical difference with the χ^2 test in a comparison of the results in the exposed and the corresponding unexposed groups

The results of this investigation suggest that no difference exists in the end results of exposed or unexposed groups of patients to the generally adopted doses of radiation treatment in painful inflammatory and degenerative conditions of the locomotor system

Addendum in proofs

A questionnaire was sent out to 368 patients seven to eight months after the 6 week control Fifteen patients could not be traced owing to change of address or death The patients were asked if there had been any change in their condition since the last control and whether they had received any further treatment Of the 353 patients who received the questionnaire 325 replied

Further treatment had been given to seventeen patients in the treated improved group they had received physio and short wave therapy but no irradiation Fifteen of the patients in the treated not improved group had had further treatment excluding irradiation In the untreated improved group sixteen patients had received further treatment excluding irradiation and in the untreated not improved group eighteen patients had received further treatment but not irradiation

The results still indicated that no differences existed as regards the degree of improvement between the treated and untreated groups and that the results had not materially altered since the 6 week control In the treated group 104 patients of the 111 improved at the 6 week control were still improved at the 8 month control of the 47 not improved at the 6 week control 21 had improved at the 8 month control In the untreated group 95 of 108 remained improved and 19 of the 59 changed their statement from not improved to improved

SUMMARY

A series of 399 patients suitably controlled and representing 439 painful locomotor ailments, was studied The conclusion was reached that no difference existed in the end results of patients exposed and those not exposed to radiation treatment for the conditions

ZUSAMMENFASSUNG

Ein Material von 399 Patienten die an 439 schmerzhaften Erkrankungen des locomotorischen Systems litten wurde kritisch überprüft Es ergab sich dass die Strahlenbehandlung das Endresultat nicht beeinflusst

FROM THE DEPARTMENT OF RADIATION BIOLOGY AND BIOPHYSICS UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY ROCHESTER NEW YORK USA

EFFECT OF SELECTIVE TUMOR HEATING ON THE LOCALIZATION OF ^{131}I FIBRINOGEN IN THE WALKER CARCINOMA 256

II Heating with microwaves

by

E S COPELAND and S M MICHAELSON

It is thought that the primary effect of microwave irradiation on mammalian tissues results from increased molecular vibration within these tissues i e microwave irradiation increases tissue temperature (MICHAELSON et coll 1961) CATER et coll (1964) have shown that microwave heating acts synergistically with radiation in destroying the rat hepatoma 223 CATER review the field of microwave radiation therapy of tumors revealing that few tumors can be destroyed by microwaves alone Their heating effect may however like warm water, be able to increase ^{131}I fibrinogen localization so that ^{131}I β radiation can destroy tumor tissue Microwaves should be nearly completely blocked by fine mesh copper wire screening (REYNOLDS 1961) Hence the body of a rat bearing the Walker tumor could presumably be satisfactorily shielded from the microwave beam and only the tumor with its overlying skin directly exposed to the heating effects of the

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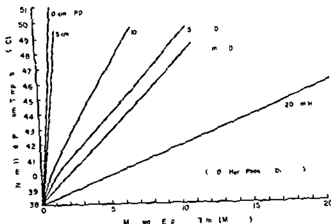


Fig 2 Microwave dosimetry phantom study The temperature of a 10 ml water phantom is plotted as a function of exposure time at various distances from the microwave transmitter wave guide horn The microwave transmitter was operating at 1 10 kV and 180 mA Approximate calculations suggest that at 15 cm HPD the effective microwave power concentration was 0 258 watt/cm

Materials and Methods

Experimental animals and materials were handled and prepared as previously described (COPELAND 1970)

Microwave irradiation technique The microwave apparatus described by MERMAGEN (1961) was used for these irradiations (*Fig* 1) Microwaves (continuous) of 10 7 cm wavelength and 2 800 MHz frequency were produced by the transmitter operating at 1 19 kV and 180 mA The irradiations were performed with the center of the tumor at a distance of 15 cm from the end of the wave guide horn i e 15 cm horn tumor distance (HTD) Phantom studies were done to determine the effective power output at various distances from the horn Ten grams of water were placed in a plastic vessel and the water temperature measured with a thermocouple as a function of irradiation time at various distances from the wave guide horn

In order to shield the rat during irradiation a 2 5 centimeter hole was cut in a fine mesh wire screen which was wrapped around a lucite anesthesia chamber The tumor was pulled outside the lucite cylinder and through the hole in the screen and thus selectively exposed to micowave irradiation (see Fig 1) In order to determine the effectiveness of the wire screen as a shielding material against

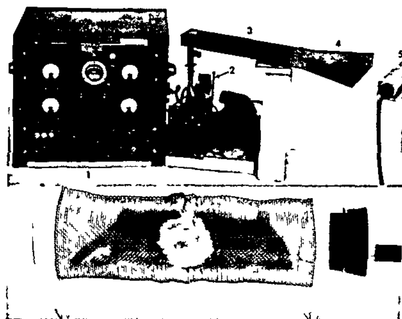


Fig 1 Microwave irradiation study apparatus *Upper view* Microwave irradiation unit (AN MPS 14 Radar set) (continuous wave) In the studies reported here the direct current power supply (1) was operated at 1 19 kV and 180 mA Microwaves were generated by the magnetron tube (2) which was cooled by the centrifugal fan shown in right center Microwaves left the magnetron tube entered a preliminary tunable wave guide traveled along a coaxial cable to a stub antenna in the proximal end of the wave guide (3) and were then regenerated they traveled down the wave guide to the horn (4) and thence to the tumor of the shielded rat (5) The microwaves had a frequency of 2 800 MHz and a wavelength of 10 7 cm (World War II S band) *Lower view* Anesthesia chamber used for tumor microwave irradiation This lucite cylinder was used as an anesthesia chamber for the microwave irradiation studies During microwave irradiation the rat s body was shielded by the fine mesh wire screen shown here As can be seen the tumor was exposed to microwaves by being pulled through a hole in the wire screen Phantom studies showed that this wire screen effectively blocked microwaves

microwave irradiation This heating then should induce sufficient tumor damage to cause increased tumor ^{131}I fibrinogen localization

BALE and co-workers have used radioiodinated human fibrinogen and anti fibrin antibody with limited success in the therapy of human tumors (DEWEY et coll 1963, MCCARDLE et coll 1966) Some human tumors, like the Walker carcinoma 256 of the rat, do not show remarkable localizing ability for fibrinogen or antifibrin antibody It is now obvious that in human cancer therapy, some method of getting greater and more rapid localization of radioactivity in tumor is needed if this technique is to be of widespread use

The experimental objective of the present research is to determine whether sufficient injury can be imparted to tumor tissue by tumor directed microwave irradiation to enhance localization of iodinated fibrinogen in the Walker tumor

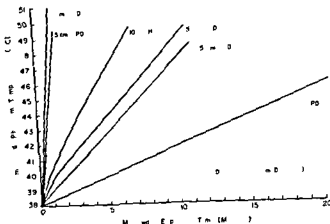


Fig 2 Microwave dosimetry phantom study The temperature of a 10 ml water phantom is plotted as a function of exposure time at various distances from the microwave transmitter wave guide horn The microwave transmitter was operating at 1 10 kV and 180 mA Approximate calculation suggest that at 15 cm HPD the effective microwave power concentration was 0 258 watt/cm

Materials and Methods

Experimental animals and materials were handled and prepared as previously described (Copeland 1970)

Microwave irradiation technique The microwave apparatus described by Mermagen (1961) was used for these irradiations (Fig 1) Microwaves (continuous) of 10 7 cm wavelength and 2 800 MHz frequency were produced by the transmitter operating at 1 19 kV, and 180 mA The irradiations were performed with the center of the tumor at a distance of 15 cm from the end of the wave guide horn i e 15 cm horn tumor distance (HTD) Phantom studies were done to determine the effective power output at various distances from the horn Ten grams of water were placed in a plastic vessel and the water temperature measured with a thermocouple as a function of irradiation time at various distances from the wave guide horn

In order to shield the rat during irradiation a 2 5 centimeter hole was cut in a fine mesh wire screen which was wrapped around a lucite anesthesia chamber The tumor was pulled outside the lucite cylinder and through the hole in the screen and thus selectively exposed to micowave irradiation (see Fig 1) In order to determine the effectiveness of the wire screen as a shielding material against

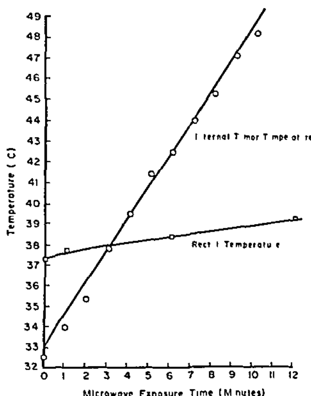


Fig 3 Effect of tumor directed micro wave irradiation on tumor and rectal temperature This nearly linear tempera ture time curve was obtained when the tumor borne by a rat was irradiated with microwaves as described in the text and the internal temperature of the tumor was followed by a thermistor probe in serted into the center of the tumor The irradiation conditions (1 19 kV 180 mA 1) cm HTD) were the same used throughout the microwave studies The body of the rat was shielded with fine mesh copper wire screen as previously described The rectal temperature plotted here was measured with a rectal therm istor probe during the course of a typi cal irradiation treatment

microwaves, the water phantom was exposed to the microwave beam and the temperature was recorded as described above Then the wire screen was inserted between the microwave source and the phantom, 4 cm from the phantom

Results

The parameters measured, percentage of injected dose per gram tumor normalized (% ID/gTN), therapeutic ratio, and percentage of whole body radio activity retained, were described in the preceding paper (COPELAND)

Microwave dosimetry In order to determine the microwave power density and heating characteristics in the various regions around the wave guide horn dosi metry studies were conducted The object of this study was to find a suitable irradiation distance, i e a horn tumor distance at which the microwave power output was great enough that the tumor could be heated to about 45° C in a pe riod of time short enough to be practical, but long enough to be easily controlled and reproduced It also had to be demonstrated that fine mesh copper screen could effectively block microwave irradiation

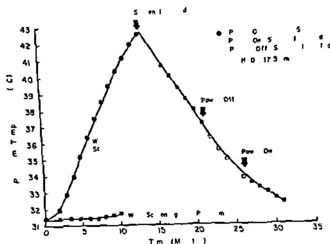


Fig 4 Effectiveness of fine mesh copper screen as a microwave shield In order to determine the effectiveness of the wire screen as a shielding material against microwaves the water phantom was exposed to a microwave beam and the temperature was recorded as previously described Then the wire screen was inserted between the microwave source and the phantom 4 cm from the phantom As indicated in the figure no matter whether the microwave source was turned off or on the phantom temperature continued to drop at a nearly constant rate about 0 7 C/min This study suggests that there is no heating effect of microwaves behind the wire screen If the power was turned on after the wire screen was inserted there was little rise in phantom temperature during ten minutes of irradiation

The rate of temperature increase of the 10 ml water phantom when placed at various distances from the wave guide horn is shown in Fig 2 At 15 cm HPD the temperature rose 5 75° C in 5 minutes It is indicated in Fig 4 that the rate at which the phantom loses heat in this temperature range is about 0 7° C/min Hence the phantom absorbed 18 5 g cal/min The phantom cross sectional area was 5 0 cm thus the effective microwave power concentration at 15 cm HPD was 0 258 watt/cm

The results of a study in which a thermistor probe was inserted into the center of a 10 day Walker tumor borne by an anesthetized and shielded rat and the internal tumor temperature was recorded as a function of the time exposed to microwaves at 15 cm HTD are presented in Fig 3 This temperature rose 15 C in 10 minutes Rat core temperature rose less than 2 C The tumor phantom temperature rose 11° C when irradiated under the same conditions (see Fig 2) Such agreement between tumor and phantom temperature response is good in view of the lightly greater cross-sectional area and lower heat loss rate of the tumor

Table 1

Effect of tumor directed microwave irradiation on the localization of ^{131}I fibrinogen in the Walker carcinoma 256

Treatment	Number of rats	%ID/gTN	Therapeutic ratio	3 day whole body radio activity retained (%)	Average rat weight (gram)	Average tumor weight (gram)
Control						
R T 10	14	0 81±0 07	8 0±0 9	19 0±1 6	138±5	15 04±1 45
M W 5	10	2 19±0 37*	27 2±5 6*	23 8±2 7	141±3	12 08±1 90
M W 10	10	3 26±0 43*	44 8±7 7*	24 9±4 4	144±3	6 97±0 93
M W 12	5	2 58±0 28*	32 6±5 0*	16 6±1 4	129±3	5 39±1 14

* Indicates a statistically significant difference from the control level at the 0 5 % significance level
R T — indicates room temperature about 25 C
M W is used here and elsewhere in this study as an abbreviation for microwave irradiation
On this and each of the following tables each parameter is followed by ± standard error of the mean The text should be consulted for a description of the experimental protocol used to obtain these data

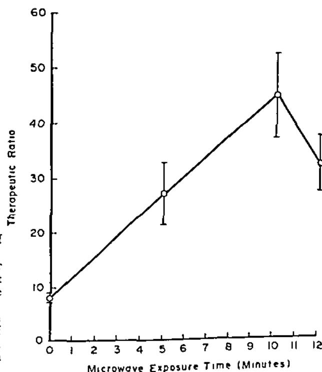


Fig 5 Effect of tumor directed microwave irradiation on the localization of ^{131}I fibrinogen in the Walker carcinoma 256 The therapeutic ratio for each of the three microwave treatment groups and the control is plotted here as a function of the microwave exposure time The vertical bar at each point represents the standard error of the mean All treatment groups showed a statistically significant difference from the control therapeutic ratio

Table 2

Kinetics of tumor fibrinogen localization after selecti e tumor microuave irradiation

Treatment	Number of rats	ID/gTN	Therapeutic ratio	Whole body radioactivity retained (o)	Average rat e ght (gram)	Average tumor weight (gram)
Control (R T 10) 24 hours	4	1 37±0 08	3 3±0 3	46 4±3 4	141±6	8 83±1 24
Control 47 hours	3	1 00±0 04	5 3±0 5	24 4±0 6	141±8	11 98±0 66
Control 67 hours	14	0 81±0 07	8 0±0 9	19 0±1 6	138±5	15 04±1 45
5 M W 21 hours	4	2 9ɔ±0 41	9 0±1 8	47 7±3 8	145±3	8 52±1 58
5 M W 47 hours	4	2 50±0 50	21 0±2 0	23 1±3 1	139±8	7 46±1 72
5 M W 67 hours	10	2 19±0 37	27 2±5 6	23 8±2 7	141±3	12 08±1 90
10 M W 21 hours	4	9 36±0 08	4 6±0 7	69 5±7 2	136±3	8 54±2 26
10 M W 41 hours	3	2 13±0 13	10 4±2 9	31 4±7 4	136±9	6 39±1 61
10 M W 62 hours	10	3 26±0 43	44 8±7 7	24 9±4 4	144±3	6 97±0 93

The text should be consulted for an e planation of the experimental protocol from which these data were obtain d All the 3-day (67 hrs) parameters reported here represent a compilation from sev ral experim nts and we e reported in Table 1

The results of a study demonstrating the effectiveness of copper wire screen as a microwave shield are presented in Fig 4 The temperature record shows that *there is no microwave* heating effect behind the wire screen

Effect of microuave irradiation on tumor fibrinogen localization Experimental animals were treated in the manner previously described (COPELAND) their tumors being exposed to microwaves at 15 cm HTD for 5 10 or 12 minutes The tumors of control animals were mock irradiated i e positioned for irradiation but given none The results of several microwave studies are compiled in Table 1 Both the parameters which measure fibrinogen localization indicated a statistically significant increase in localization above the control level The ratios of experimental therapeutic ratio to control therapeutic ratio are 3 4 5 6 and 5 1 for the 5 10 and 12 minutes microwave exposure times respectively The therapeutic

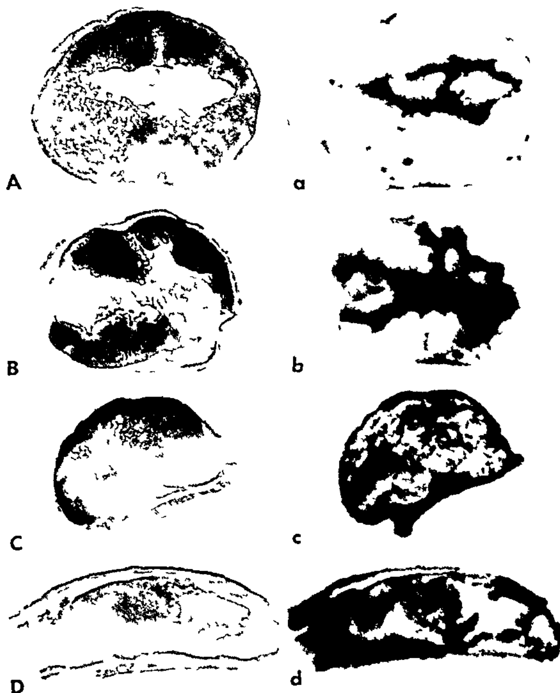


Fig 6 Photomicrographs and radioautographs of tumors selectively irradiated with microwaves 3 day uptake magnification ×1 6

A—a) Control tumor (R T 10) This tumor has a moderately sized pink stained medullary region and a large dark blue stained cortex It is similar in all respects to previously described 3 day control tumors Radioactivity is located primarily in the pink medulla

B—b) Treated tumor (Microwaves …) An enlarged pink stained medullary region is present in this tumor Radioactivity is found in this region The kinetic study reveals events which precede this third day localization pattern

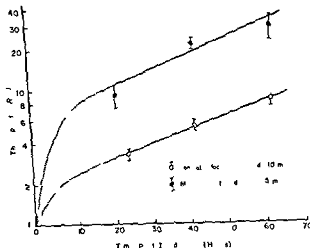


Fig 7 Kinetics of ^{131}I fibrinogen localization in the Walker carcinoma 256 after selective microwave irradiation of the tumor Therapeutic ratio is plotted as a function of time between initiation of treatment and sacrifice for control and five minute microwave irradiation the vertical bars at each point indicate the variation allowed by the standard error of the mean In each group the therapeutic ratio seems to increase exponentially with time after about 20 hours post irradiation All data plotted here were taken from Table 2 Curves to the left of the one day values are dotted to indicate that they are hypothetical

ratio is plotted against microwave exposure time in Fig 5 Representative stained sections and the corresponding radioautographs are presented in Fig 6 In general as the microwave exposure time is increased, the fraction of the tumor which is necrotic increases and the radioautograph density increases correspondingly However at the 5 minutes exposure level radioautograph density is not limited to necrotic tumor tissue but encroaches into viable tissue zones Although the 10 and 12 minutes exposed tumors do not appear to be completely necrotic ^{131}I activity is distributed more or less evenly throughout the tumor Other tumor sections not presented here reveal considerable tumor erosion at the 10 and 12 minutes exposure levels

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C—c) Treated tumor (Microwaves 10) This tumor does not show a characteristic medullary region It seems to contain more vascular components than A—a or B—b The whole section is stained pinkish blue Radioactivity is deposited throughout the tumor

D—d) Treated tumor (Microwaves-12) Like the preceding tumor section no medullary region as such is present here The section is pink in gross appearance but contains large regions of otherwise normal tumor cells The one region not containing radioactivity has a band composed of cells with fragmented nuclei Considerable tumor erosion was evidenced in other members of this group

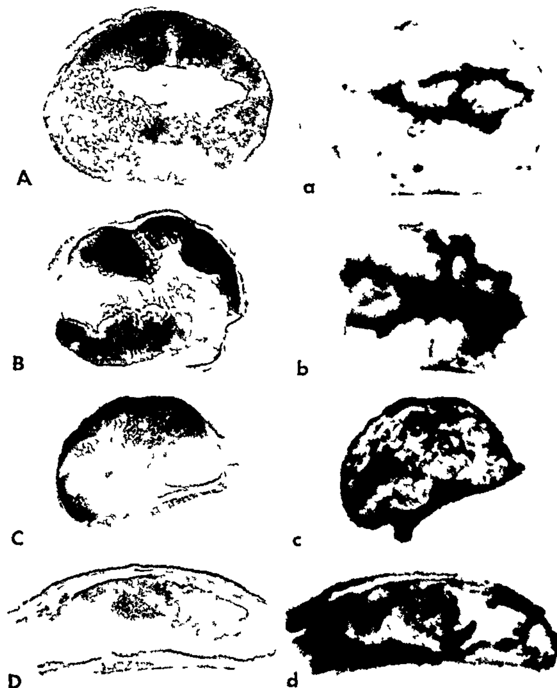


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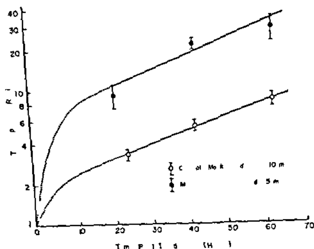


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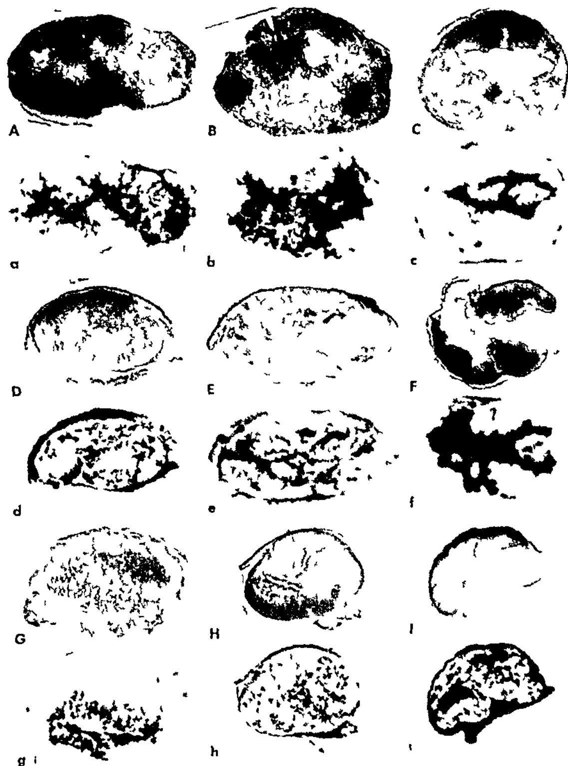


Fig. 8 (For legend see opposite page.)

Kinetics of tumor fibrinogen localization after microwave irradiation The following experiment was performed to determine the time sequence of fibrinogen localization in microwave irradiated tumors Rats were divided into three treatment groups and their tumors were either mock irradiated (controls) or exposed to microwaves for 5 minutes or for 10 minutes The irradiation conditions were identical to those used in the 3 day uptake study Animals from each of the three groups were sacrificed on each of the three days following treatment The data obtained from this study are presented in Table 2 The variation of the experimental and control therapeutic ratios as functions of time are plotted in Fig 7 Representative stained sections and the corresponding radioautographs are presented in Fig 8 In the control tumors ^{131}I activity is found in necrotic tumor tissue At the 10 minutes microwave exposure level ^{131}I activity is found in the necrotic tumor medulla on the first day but is located throughout the tumor by the third day post treatment

Discussion

It has been found that when the tumor is exposed to 2 800 MHz (CW) 0 258 watt/cm microwaves for five minutes localization of ^{131}I fibrinogen within the tumor is more than doubled for the next 3 days when compared to that in control tumors which were positioned for irradiation but given none

Kinetic studies carried out with the five minute microwave treatment group showed that tumor radioactivity reached its maximum within 24 hours after treatment and remained high for at least 3 days These studies also suggest that the therapeutic ratio increased exponentially with time after about 20 hours following treatment initiation A theoretical analysis of tumor fibrinogen locali

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Fig 8 Photomacrographs and radioautographs *from* kinetics of uptake study after microwave irradiation of tumor magnification ×1

A—a) B—b) and C—c) Control tumors (R T 10) *1 2 and 3* days Radioactivity remains localized in the variously shaped tumor medulla These control tumors have all the characteristics previously described for untreated *tumors* *There* is no meaningful change in the pattern of radioactivity localization with time

D—d) E—e) and F—f) Treated tumors (*Microwaves* 5) *1 2* and 3 days On the first and second days after treatment (D—d and E—e) the tumor sections have a generalized pinkish [illegible] and do not have a characteristic *medullary* region The third day section (F—*f*) *has a* characteristic medulla Radioactivity *seems* widespread but highest in the cortical regions on day [illegible] still widespread but more concentrated in the central regions on day 2 and definitely *localized* to the tumor medulla on day 3

G—g H—h) and I—i) Treated tumors (Microwaves 10) 1 2 and 3 days A definite medullary region is evident *on the first* day after treatment but is not evident thereafter On the first day radioactivity is limited to *the pink medullary* portion On the second day radioactivity has become widespread throughout the pinkish blue *tumor section* *By* the third day the radioactivity has *become* quite concentrated throughout the tumor

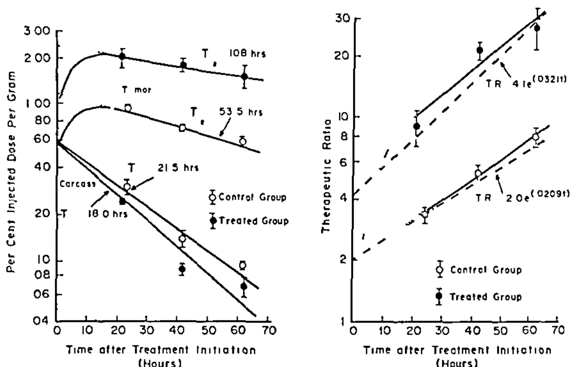


Fig 9 Theoretical analysis of change in therapeutic ratio with time *Left* Specific activity of tumor and carcass is plotted versus time after treatment and the biological half lives of ^{131}I fibrinogen (T½) are determined *Right* Experimental (solid lines) and calculated (dashed lines) therapeutic ratios are plotted versus time

zation after five minutes of microwave irradiation is presented in Fig 9 On the left of the figure, the logarithms of percentage of injected dose per gram (% ID/g) of irradiated and control tumor tissue and for the carcasses of treated and control rats are plotted against time These curves are consistent with the hypothesis that *^{131}I fibrinogen is deposited in the tumor primarily during the* first 20 hours after injection whether the tumor is irradiated or not, and that thereafter tumor radioactivity concentration decreases exponentially with time

The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor The initial carcass radioactivity concentration is approximately the same in the treated and control groups and the carcass biological half life of ^{131}I fibrinogen is almost the same in the two groups

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation, $A_t = A_0 e^{-\lambda t}$, as in the previous study (COPELAND)

When the suitable constants determined in Fig 9 are substituted into this rela

tion the therapeutic ratio as a function of time for control and treated groups can be calculated

$$T R_{control} = 2.1\, e^{0.193t} \quad T R_{treated} = 4.1\, e^{0.09t}$$

These calculated expressions for the therapeutic ratios are plotted as functions of time on the right side of Fig 9 The variation of the experimental therapeutic ratios with time are also plotted here as they were in Fig 7 The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference in biological half life of ^{131}I fibrinogen in the tumor and carcass.

The effect of microwave treatment on total ^{131}I radiation doses to tumor and carcass can be calculated as in the previous paper

Thus

$$\frac{\text{average beta dose to tumor}}{\text{average beta dose to carcass}} = \begin{array}{l} 4.1 \times 4.19 = 17.2 \text{ for treated group} \\ 2.0 \times 2.17 = 4.3 \text{ for control group} \end{array}$$

This calculation indicates that five minute microwave irradiation improved the ^{131}I fibrinogen radiation therapy of the Walker tumor nearly 400 per cent In tumors whose positions can be reasonably well determined as by ^{131}I antifibrin antibody (McCARDLE et coll) or classical diagnostic techniques tumor directed microwave irradiation seems an effective means to increase ^{131}I fibrinogen deposition to levels high enough for therapy As shown previously (COPELAND) normal tissues in the tumor environment should not be damaged by heating

Acknowledgement

This work is part of a Ph D thesis in Radiation Biology at the University of Rochester and was supported in part by a National Institutes of Health training grant in biophysics by RADC Contract AF30 (603) 224 U S Air Force dated 1 March 1958 and by Contract W 7401 Eng 49 U S Atomic Energy Commission The publication has been assigned Report No UR 149 126f

SUMMARY

Heating of Walker carcinoma 256 by selective irradiation with 2800 MHz, 0 2-8 watt/cm microwaves for five minutes induced a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which will localize in the tumor In irradiated tumors radioactivity was widely distributed and seemed to coincide with histologically demonstrable areas of tumor damage This tumor heating technique could potentially increase tumor radiation therapy dose from ^{131}I fibrinogen 400 per cent

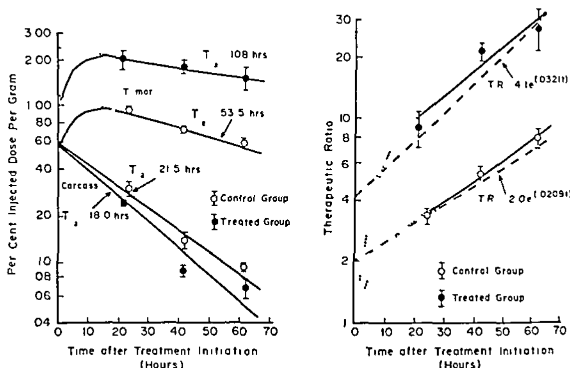


Fig 9 Theoretical analysis of change in therapeutic ratio with time *Left* Specific activity of tumor and carcass is plotted versus time after treatment and the biological half lives of ^{131}I fibrinogen ($T_{1/2}$) are determined *Right* Experimental (solid lines) and calculated (dashed lines) therapeutic ratios are plotted versus time

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The carcass, on the other hand, suffers an exponential loss in radioactivity concentration from the time of injection and this loss occurs at a greater rate than in the tumor The initial carcass radioactivity concentration is approximately the same in the treated and control groups and the carcass biological half life of ^{131}I fibrinogen is almost the same in the two groups

The straight line portions of the log % ID/g versus time plots can be assigned mathematical descriptions based on the exponential relation, $A_t = A_0 e^{-\lambda t}$, as in the previous study (COPELAND)

When the suitable constants determined in Fig 9 are substituted into this rela

tion the therapeutic ratio as a function of time for control and treated groups can be calculated

$$TR_{control} = 2\ 1\ e^{0193t},\ TR_{treated} = 4\ 1\ e^{0\ 09t}$$

These calculated expressions for the therapeutic ratios are plotted as functions of time on the right side of Fig 9 The variation of the experimental therapeutic ratios with time are also plotted here as they were in Fig 7 The good agreement between calculated and observed therapeutic ratio versus time curves is evidence that the exponential increase of therapeutic ratio with time depends on the difference in biological half life of ^{131}I fibrinogen in the tumor and carcass.

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Acknowledgement

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SUMMARY

Heating of Walker carcinoma 256 by selective irradiation with 2800 MHz 0 258 watt/cm microwaves for five minutes induced a substantial increase in the amount of intravenously injected ^{131}I fibrinogen which will localize in the tumor In irradiated tumors radioactivity was widely distributed and seemed to coincide with histologically demonstrable areas of tumor damage This tumor heating technique could potentially increase tumor radiation therapy dose from ^{131}I fibrinogen 400 per cent

ZUSAMMENFASSUNG

Eine Erwarmung des Walker Carcinoms 256 durch selektive Bestrahlung mit 2 800 MHz, 0 258 Watt/cm² Mikrowellen 5 Minuten lang fuhrt zu einem kraftigen Anstieg der Menge intravenos injizierten [131]I Fibrinogens die sich im Tumor ansammelt In bestrahlten Tumoren ist die Radioaktivitat weit verteilt und scheint mit der histologisch nachweisbaren Ausbreitung der Tumorschadigung ubereinzustimmen Diese Warmebehandlung des Tumors konnte moglicherweise die therapeutische Strahlendosis des Tumors durch [131]I Fibrinogen um 400 % erhohen

RÉSUMÉ

L elevation de temperature dans un carcinome 256 de Walker par irradiation selective de micro ondes de 2 800 MHz avec une puissance de 0 258 watt/cm² pendant cinq minutes augmente de façon importante la fixation dans la tumeur du fibrinogene marque par [131]I *injecte par voie veineuse Dans les tumeurs irradiees* la radio activite etait largement dispersee et paraissait coincider avec des zones ou l histologie montrait les lesions causees a la tumeur Cette technique de chauffage des tumeurs pourrait peut etre quadrupler la dose de radiotherapie fournie par le fibrinogene marque par [131]I

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FROM THE DEPARTMENT OF TUMOR BIOLOGY, THE MEDICAL SCHOOL OF KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

MULTIPLICATION OF TUMOR CELLS IN VITRO AFTER OXIC OR ANOXIC EXPOSURE TO ROENTGEN RADIATION

by

Bo LITTBRAND

The oxygen gradients in tumours vary. The radiation response of more or less hypoxic cells has consequently often been investigated with a view to improving the control of these cells by therapeutic irradiation. The study of radiation damage to mammalian cells grown in vitro represents a frequently practised experimental approach. The damage has been measured either by the inhibition of single cells to form colonies or by the inhibition of the growth of the cell populations. In the former instance data have accumulated on the reactions to single or multiple radiation exposures under oxic, hypoxic and anoxic conditions (ELKIND & WHITMORE 1967). However, while many articles have dealt with the effect of single oxic radiation exposures with growth inhibition as a criterion for the damage, few and incomplete data are available on the effect of multiple oxic exposures or exposures in anoxia.

The purpose of the present experiments was to obtain information on the multiplication of cells irradiated with a single dose or the same dose split into two fractions in the presence or absence of oxygen. The growth curves will be analysed with regard to the clonogenic survival of the cells. Cultures of the ELD

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ascites tumour and several of its substrains with different radiosensitivity were therefore chosen as the biologic material for which data on the oxic and anoxic survival are available from previous studies (REVESZ & LITTBRAND 1964 and 1967).

Material and Methods

Cell cultures. Cultures of a hyperdiploid Ehrlich ascites tumour, denoted ELD, and four of its substrains denoted ELT, SELD clone a, SELD clone f and SELD clone g were used. The isolation of the clones, the culture method in vitro and the characteristics of these strains have been described (HAUSCHKA et coll. 1957, LITTBRAND 1970, MODIG 1968, REVESZ et coll. 1963, 1964). The mean plating efficiency of the different strains varied between 40 and 80 %. The ELT substrains have a duplicated, i.e. hypertetraploid chromosome set in relation to ELD and characterized by an increased radioresistance in in vitro and in vivo conditions (GLAS & REVESZ 1963, LITTBRAND 1970, REVESZ & LITTBRAND 1964). The cells of SELD clone a, clone f and clone g have chromosome sets in the same ploidy region as ELD but differ in their sensitivity to radiation both in vivo and in vitro (LITTBRAND 1970, REVESZ et coll. 1963, 1964).

Experimental design. Suspension of monodispersed cells were prepared from 6- to 8-day old cultures grown in a monolayer on the surface of stoppered milk dilution bottles. The cells were readily dissociated by treatment with 0.5 % trypsin solution at a temperature of 37° C for 10 minutes. The cells in the number required were explanted in a series of 6 cm pyrex petri dishes containing 4 to 6 ml of nutrient medium after their concentration had been established in a haemocytometer. The culture medium consisted of Eagle's medium in Earle's solution (EAGLE 1959) and was supplemented with 15 % fetal calf serum and antibiotics. The cultures were incubated at 37° C in a moist atmosphere of air with 5 % carbon dioxide added to maintain a pH of 7.0 to 7.2. The cells after 4 days incubation were exposed to radiation and thereafter re-incubated, with a medium change every second day. The number of cells in the population was determined after periods varying from 3 to 21 days. The cells were brought into suspension by exposure to trypsin and by scraping the surface of the dishes with an edged teflon rod. They were counted by a Coulter Model B electronic particle counter. The mean value from duplicate dishes was calculated routinely.

Radiation exposure and control of atmosphere. The exposure to roentgen radiation was at a temperature of about 27° C in an air tight plastic chamber of 2 l volumes, with two orifices of 6 mm diameter for the inlet and outlet of

gases The lids of the culture dishes were removed and the nutrient medium withdrawn so that not more than 1 ml fluid was left to cover the cells The chamber was filled with either oxygen or argon with 1 75 % carbon dioxide at a flow rate adjusted to 6 l/min The gases passed through a pre-equilibrated water column warmed to a temperature of 38° C Argon with an oxygen contamination of less than 3 ppm was used and was led to the cells through a system of stainless steel and glass tubes with joints sealed impermeably to oxygen from the atmosphere The outflowing gas passed a water lock and was routinely monitored by an Elcoflux oxygen analyser (Dr Thiedig & Co KG Berlin) The flushing period of the chamber with the respective gases was 11 minutes when a dose of 1 100 R in argon or 450 R in oxygen was delivered or when these doses were given in two fractions When irradiation was made with 2 500 R in argon or 1 000 R in oxygen or with two fractions of these doses the total flushing period was 17 minutes. Irradiation was always adjusted to terminate at the end of the flushing period As has been reported elsewhere (LITTBRAND 1970) a 4 minute flushing was found to be sufficient for equilibration between the fluid and the gas phase under the conditions described

The dishes after exposure to radiation were refilled with 5 ml fresh medium before incubation Two exposures were made at 4 or 18-hour time intervals as described in the experiments with split doses the cells were incubated aerobically during the intervals between the doses The cells that were irradiated *with the total* dose at the first exposure were sham irradiated at a time corresponding to the second exposure of the split dose treated cells Unirradiated control cultures were also sham irradiated under the same conditions as the irradiated cells

Radiation factors Radiation was generated in a Siemens roentgen unit at 190 kV and 15 mA with 1 5 mm Al inherent filtration and 1 0 mm Al additional filter half value layer 0 95 *mm* Cu The dose rate was 235 R/min at the bottom of the culture dishes which were always placed at a distance of 43 cm from the focus The dose delivered was measured by a Philips integrating dosimeter In all the tests the cells when irradiated were attached to the glass surface The correction factor *for backscatter* as determined from survival measurements with Chinese hamster cells while attached to glass versus plastic, was 1 35 ± 0 05

Results

The population growth of ELD was studied after exposure to roentgen radiation either in a relatively low dose range (450 R delivered in oxygen or

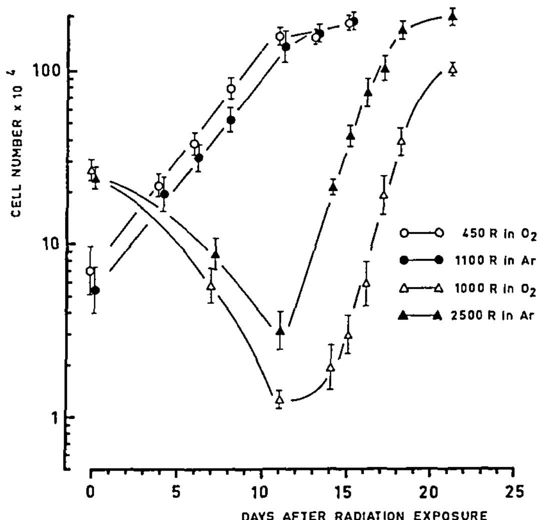


Fig 1 Growth of ELD ascites tumour cell cultures after irradiation in argon or oxygen Mean ± S E of the cell number is indicated

1 100 R in argon) or in a large dose range (1 000 R in oxygen or 2 500 R in argon) Treatment was made either with the total dose at a single exposure or with the dose split into two fractions delivered 4 or 18 hours apart

Cell populations that originated from an inoculum of 0 3 × 10^4 cells and reached a size of about 8 × 10^4 cells after 4 days of growth were irradiated with the doses in the lower range For exposures with the doses in the higher range populations were used that originated from an inoculum of 10^4 cells and contained about 25 × 10^4 cells after 4 days of proliferation when the irradiation was administered As the plating efficiency of ELD in these experiments was 80 ± 4 % (mean ± S E), it can be calculated that the mean doubling time

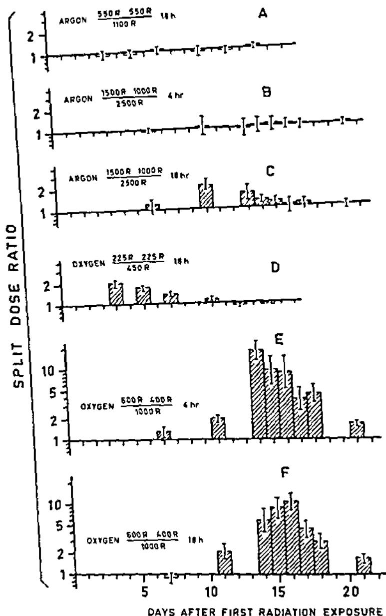


Fig 2 Split-dose ratios of ELD cells defined as the ratios between the population size at corresponding times after treatment with a single dose and the same dose delivered in two fractions The irradiation was under either oxic or anoxic conditions The split dose interval was 4 or 18 hours during which period the cells were incubated aerobically Columns indicate the mean ± S E of the split dose ratios

of the population before irradiation was about 20 5 hours The value varied within the range of 18—24 hours in the individual experiments

The mean population sizes at different times after single radiation exposures are given in Fig 1 When the doses in the low range were given in a series of 10 repeat oxic and in the same number of anoxic experiments, the cell population increased exponentially until it reached a mean number of about 10^6 cells The mean population doubling time during this growth phase was about 56 hours The growth then rapidly decelerated and the population approached a maximum level of 2 $\times$ 10^6 cells

The mean population size decreased gradually in an initial phase when in 13 repeat experiments in argon and in 16 experiments in oxygen the cell populations were exposed to the doses in the high range Eleven days after treatment in argon and fourteen days after treatment in oxygen the populations started to increase exponentially with a doubling time of 22 hours, i e with a doubling time similar to that of the cells before irradiation After the exponential phase, the growth decelerated and the cell number approached a value of about 2 $\times$ 10^6

Split dose ratios defined as the ratio between the population size at corresponding times after treatment with a single dose and the same dose delivered in two fractions are illustrated in Fig 2 In the case of anoxic irradiation in the low dose range and an 18 hour interval between the split doses (Fig 2 A), the ratios are close to unity Similar results were obtained when split doses in the larger range were given anoxically 4 hours apart (Fig 2 B) Ratios higher than unity were calculated with larger split doses under anoxic conditions and a time interval of 18 hours (Fig 2 C)

The split dose ratios obtained with irradiations under oxic conditions were significantly larger than unity Either split doses in the lower range, 18 hours apart, or in the larger range, 4 or 18 hours apart, were delivered In the former case, the ratios had a maximal value of 2 (Fig 2 D) and in the latter cases (Fig 2 E F) the ratios had a maximal value of about 20

The relative radiosensitivity of FLD and its four substrains (ELT and SELD, clones a, f and g) was studied in repeat experiments by radiation exposure of populations of each strain when they reached a size of 6—10 $\times$ 10^4 cells four days after explantation of an inoculum containing 0 3 $\times$ 10^4 cells Growth inhibition of the population was taken as the criterion of sensitivity, as indicated by the ratio between the total cell number of the sham irradiated and the irradiated cell population at corresponding times determined in the same experiment The doubling time of the sham irradiated substrains was within the same range as the parental strain Exposure doses of 450 R delivered in oxygen or 1 100 R in argon were always employed The results obtained in 5

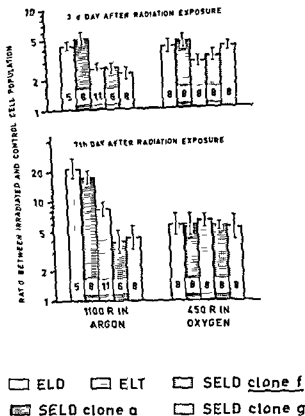


Fig 3 Growth inhibition of ELD and its four substrains as indicated by the ratio between the total cell number of sham irradiated and irradiated populations at corresponding times Doses of 1100 R in argon or 450 R in oxygen were delivered Columns show mean ± S E calculated from repeated experiments in numbers indicated by the figures inside the columns

to 11 repeat experiments 3 and 7 days after irradiation are illustrated in Fig 3 The growth of ELD and clone a is inhibited by irradiation in argon to a greater degree than the other cell strains As calculated by covariance and t test analysis the differences are significant in all cases ($p < 0.01$ or < 0.05) In contrast irradiation in oxygen inhibits the growth of all the cell strains to a similar extent and differences are statistically insignificant

Discussion

The cell population at different times after irradiation may be regarded as being composed of cells which either retained reproductive integrity or lost their capacity for unlimited reproduction after suffering lethal damage We

attempted to calculate the proportion of the two types of cells in the populations presented in Fig 1 by estimating (1) the fraction of cells that retained reproductive integrity, (2) the growth rate of these cells and (3) their division delay

Analysis of the growth curves after oxic irradiation After an exposure dose of 1 000 R, clonogenic cells can be expected to survive in a proportion 4×10 to 10^{-4} if it is assumed that the survival curve of these cells has an extrapolation number between 2 and 5 and a $D_0 = 91$ R (REVESZ & LITTBRAND 1964, and 1969) Accordingly, only about 10 to 25 cells can be expected to retain reproductive integrity in the population irradiated with 1 000 R in our tests Lethally damaged cells may therefore be considered to constitute the great majority of the population for several days after irradiation The decrease of the population, as seen in Fig 1, reflects obviously the rate of disintegration of these cells The finding that only 5 % of the original population was left 11 days after irradiation is in agreement with the observation by TOLMACH (1961) on HeLa cells exposed to a comparable dose

The exponential increase of the population starting 14 days after irradiation is conceivably due to the growth of survivors that retained reproductive integrity During the exponential growth, the population has an average doubling time of 22 hours, which differs slightly from the doubling time of 20 5 hours of the unirradiated cells By extrapolating the exponential part of the growth curve to the day of irradiation, and assuming a division delay of 1 min/rad (ELKIND & WHITMORE 1967, SACHER 1968) corresponding to 23 hours, it can be calculated that on an average a single cell is left in the irradiated population with unaltered growth rate Since the number of clonogenic cells is larger by a decade (cf previous paragraph), it may be concluded that most of the survivors have a considerably lower growth rate This conclusion is in agreement with considerations (DEWEY et coll 1963, NIAS & FOX 1968) according to which the clonogenic cell estimate, arrived at by counting colonies with a minimum of 50 cells, comprises cells that have a prolonged generation time Obviously, such cells can constitute only a negligible fraction of the exponentially growing population 14 days or more after irradiation

When divided doses were delivered, the split dose ratios during the exponential growth phase had a mean value of 8, either 4 or 18 hours being the interval between the doses These split dose ratios are considerably larger than those that have been calculated when the clonogenic capacity was used as criterion of survival and when mean values 2 (plateau phase cells) and 4 (exponentially growing cells) (REVESZ & LITTBRAND 1967 and 1969) were found The difference may be interpreted as indicating that repair during the split dose interval

concerns not only radiation damage which affects clonogenic capacity but also damage that leads to a prolongation of the generation time and possibly also to a delay of the division

An extrapolation of the exponential part of the growth curve to the 7th day after irradiation indicated that during the first seven days the great majority of the population consists of lethally damaged cells if the effect of either single or split doses is considered As indicated in Fig 2 the split-dose ratios calculated at the 7th day do not differ significantly from unity According to ELKIND et coll (1963) cells damaged lethally by a single dose of 1 000 R under aerobic conditions may divide 0 8 times on an average Due to fractionation of the dose the capacity of such cells for division does not seem to increase or the increase may be too small to be demonstrable by the methods used

Considering the number of surviving cells and their proliferation rate as being similar to unirradiated cells, it is conceivable that the great majority of the population 11 days after irradiation with a single dose of 1 000 R will still consist of lethally damaged cells However after treatment with the same dose divided in two fractions and assuming a mean split dose ratio of 8 a great portion of the population may already by the 11th day consist of rapidly proliferating cells The split dose ratio of about 2 found by this time (cf Fig 2) may therefore be attributed to the recovery of sublethally damaged cells rather than an increase in the capacity of the lethally damaged cells for division

After an exposure of 450 R the proportion of the clonogenic cells may be estimated to be about 1 8 to 4 5 % Accordingly, about 1 2 to 3×10^3 cells may be expected to retain reproductive integrity in the original population The observation that the growth curve reached plateau level after 8 days of cell proliferation and before or on the 11th day may help to estimate the number of survivors that have a similar generation time as unirradiated cells Assuming a division delay of 1 min/rad and considering a doubling time of 20 5 hours it may be calculated that the number of surviving cells with an unaltered proliferation rate is in the range 0 4—2 6×10^3 immediately after irradiation This range is lower than that of the number of clonogenic cells as can be estimated from the survival curves As discussed above the difference is conceivably due to the counting of clones with a minimum of 50 cells in the clonogenic test i e including survivors with prolonged generation times In view of this approximation of the survival ranges it may be concluded that most of the population (80 to 100 %) four days after irradiation consists of cells that have lost reproductive integrity No safe estimate of the proportion of such cells can be made during the subsequent phases of the growth curve The lethally damaged cells will constitute a gradually decreasing portion of the total population due to their disintegration and the simultaneous proliferation

of surviving cells The average doubling time of 56 hours of the population may therefore be considered to be due to the composite kinetics of cell gain and cell loss

Since surviving cells constitute a minor fraction of the population during the first four days after irradiation, the increase in the population during this period may be attributed to the proliferation of the lethally damaged cells It may be calculated from the population increase by a factor of 3 1 that these cells are capable to complete, on an average, a 1 6 division During the subsequent growth period, they may be capable of further divisions, the number of which cannot however be estimated from the data with any confidence The value 1 6 is in reasonable agreement with the observation by ELKIND et coll (1963) when Chinese hamster cells were exposed aerobically to a comparable dose of 600 rad

Also when the 450 R was delivered in two fractions, the population reached plateau level during the period between 8 and 11 days after treatment The number of clonogenic cells with a growth rate similar to the unirradiated cells can be assumed therefore to be also within the range 0 4—2 6 $\times 10^3$, as discussed above Consequently, also in this case the lethally damaged cells will constitute the great majority of the population (90 to 100 %) during the first four days following irradiation By the fourth day their number increased by a factor of 6 2 This indicates that on an average 2 6 cell divisions occurred In comparing this number with the number of divisions after a single exposure to the same total dose, it may be concluded that, due to fractionation, an additional division took place Repopulation during the fractionation interval by a factor of 2 can be excluded as an explanation in view of the result of complementary experiments performed to test this possibility In those experiments the size of a population originating from 0 3 $\times 10^4$ cells was determined, on the one hand, after four days of growth and, on the other hand, after four days of growth followed by oxic irradiation with 225 R and an additional interval of 18 hours The mean ratio between the two population sizes, as determined in 10 repeat experiments, was 0 97 $\pm$ 0 16 *This indicates that the population size does not change during* the time interval between the split doses used in the experiments

This result is in agreement with what can be expected when considering the following points

1 The doubling time of the cells extends the time interval between the split doses even without taking division delay into account

2 Repopulation, if it should occur, would increase the fraction of clonogenic survivors and consequently the growth curve could be expected to reach the plateau phase earlier than when the single total dose is given this was however not the case, as indicated by the data in Fig 2 B

3 The split dose ratios after oxic irradiation with a total dose of 1 000 R are similar when either 4 or 18 hours separated the doses (cf Fig 2 E F) if repopulation occurred, the ratios after the latter interval ought to be increased

4 When irradiation is performed under anoxic conditions with 1 100 R which reduces the number of survivors to a similar level as oxic irradiation with 450 R the population size after treatment with split doses separated by an interval of 18 hours is not increased in comparison to the population size after a single treatment with the same total dose obviously repopulation would have increased the size (cf discussion below and Fig 2 A)

Thus since repopulation during the fractionation interval may be excluded as an explanation a certain repair of the radiation damage of the lethally injured cells may be considered to account for the increase of the population treated with split doses The repair would increase the capacity of the lethally damaged cells for limited reproduction permitting the cells to complete an additional division This interpretation implies that the repair of the radiation damage which results in an increased reproductive capacity of cells may proceed not only in sublethally but also in lethally injured cells In confirmation of the suggestion by ELKIND et coll (1967) it can be concluded that lethal and sublethal cellular damage may be qualitatively similar and may differ from each other only in degree In considering the finding that no repair of the reproductive capacity of sublethally irradiated cells was demonstrable when a total exposure dose of 1 000 R was delivered it would seem that the repair mechanism which concerns this damage may itself be radiosensitive

Anoxic growth curves and the oxygen enhancement of the radiation effect The growth curve of the cell population after the exposure dose of 1 100 R in argon is similar to the growth curve obtained when the exposure dose was 450 R in oxygen (cf Fig 1) This indicates that the proliferation of the lethally damaged cells during an initial period after irradiation when they constitute most of the population, is affected to the same extent in both cases and consequently the oxygen has a dose modifying effect indicated by the ratio between the doses i e a factor 2 44 Since the growth curves of the oxically and anoxically irradiated populations reach plateau level at about the same time after irradiation it may be concluded *furthermore* that oxygen has a similar dose modifying effect with regard to the number of the survivors that retain reproductive integrity as with regard to survivors that preserve an unaltered growth rate

When the growth curve after 2 500 R delivered in argon is compared to the growth curve after 1 000 R delivered in oxygen it is apparent that the exponential phase in the former instance starts about three days earlier By

extrapolation of the exponential curve to the time of irradiation, it may be calculated that ~ 10 cells may have survived the anoxic treatment with reproductive integrity and unaltered growth rate. Considering the anoxic survival curve of these cells with a D_0 235 R and an extrapolation number 1, the number of clonogenic survivors may be estimated to be about the same.

The difference between the growth curves when irradiation is administered with 2 500 R and 1 000 R under anoxic and oxic conditions, respectively, indicates that the dose modifying effect of oxygen is expressed by a larger enhancement factor than the ratio between the doses delivered, i.e. 2.5. From the estimate of the surviving fractions, arrived at by extrapolation of the exponential part of the growth curves, a value of about 3 may be calculated as an approximate factor for the oxygen enhancement. This value is larger in comparison to the value that was calculated when the higher survival level was considered (cf. previous paragraph). A smaller oxygen enhancement factor, corresponding to a larger survival level, is in agreement with the previous observation in the experiments in which clonogenic capacity was the criterion of the radiation effect, and in which the corresponding enhancement factors may be estimated to be 2.2 and 2.5 (REVESZ & LITTBRAND 1964). The dependency of the enhancement factors upon the survival level may be regarded as a consequence of the difference between the extrapolation number of oxic and anoxic radiation survival curves, as discussed previously.

Apparently the enhancement factors calculated from the data of the growth curves are larger than the corresponding factors arrived at from the data of the clonogenic survival curves. If further experiments prove the significance of those differences, a possible explanation may be a differential oxygen enhancement of the radiation damage that concerns retardation of the growth and the damage that concerns clonogenic capacity. In order to test this possibility in current experiments the differences between the colony size distribution of oxically and anoxically irradiated clonogenic cell populations are being studied.

When radiation exposures were made under anoxic conditions, the split dose ratios were either close to unity (cf. Fig. 2-A and 2 B) or varied between 1 and 2 (cf. Fig. 2 C). Previous experiments have indicated that the clonogenic capacity of the cells fails to recover when oxygen is absent during irradiation (LITTBRAND, 1970, LITTBRAND & REVESZ 1969). As discussed above little repopulation can occur during the particular fractionation intervals after the doses employed. The increased split dose ratios indicated in *Fig. 2 C* may therefore be interpreted as indicating a certain repair which conceivably concerns the damage resulting in growth retardation and, possibly, division delay. A similar interpretation may be given also to the increased split dose ratios found after oxic irradiation (cf. Fig. 2 E and 2 F). If this interpretation is correct, it may

be concluded that the recovery of the damage leading to growth retardation and division delay is less oxygen dependent than the recovery of the clonogenic capacity In view of the standard error of the determination indicated in Fig 2 A and 2 B such a recovery in the growth retardation cannot be excluded completely either in these anoxic experiments

Sensitivity differences between cellular substrains In view of the fact that ELD and its four substrains (ELT SELD clones a f and g) used in the present experiments have a similar growth rate the data presented in Fig 3 indicate that sensitivity differences do exist between the strains when growth inhibition is the criterion of sensitivity ELT clones f and g appear to be more resistant to anoxic radiation than the parental line or clone a As discussed above it may be assumed that the overwhelming majority of the cell population three days after exposure to 1 100 R under anoxic conditions consists of cells that suffered lethal radiation damage The difference in the ratio between the size of the irradiated and control cell population during the first three days is thus due to a difference in the proliferative capacity of the lethally damaged cells It may be calculated that while lethally damaged ELD and clone a cells completed on a average 1 6 and 1 0 divisions respectively, the ELT clones f and g cells divided about 2 3 times

Seven days after the anoxic irradiation the populations consist conceivably of mixed lethally damaged and proliferating surviving cells In comparison to ELD the populations of ELT clones f and g cells are larger by a factor of 2 4 4 7 and 4 5 respectively The larger population sizes may now be interpreted as being due partly to the increased proliferative capacity of the lethally damaged cells and partly to the greater number of clonogenic survivors From the data obtained in a previous investigation in which the clonogenic survival of ELT and clone g cells were studied it may be calculated that a population 2 3 and 2 8 times larger than ELD respectively can be expected to survive after anoxic exposure to 1 100 R (REVESZ & LITTBRAND 1967 LITTBRAND 1970) these values are in a good agreement with the corresponding values indicated in Fig 3 The observation that the population size of clone a does not differ significantly from ELD after anoxic irradiation is also in agreement with the earlier finding that the clonogenic survival of these two strains is similar

As previously mentioned the sensitivity differences can be associated with the difference in ploidy in the case of ELT, which has a duplicated chromosome set and the difference in the content of intrinsic acid soluble sulphydryl compounds in the case of clones f and g in which the concentration is larger by a factor of about 1 3 relative to ELD (LITTBRAND 1970) Clone a has the same concentration of sulphydryl groups and the same chromosome number as ELD

extrapolation of the exponential curve to the time of irradiation, it may be calculated that ~ 10 cells may have survived the anoxic treatment with reproductive integrity and unaltered growth rate Considering the anoxic survival curve of these cells with a D_0 235 R and an extrapolation number 1, the number of clonogenic survivors may be estimated to be about the same

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Apparently the enhancement factors calculated from the data of the growth curves are larger than the corresponding factors arrived at from the data of the clonogenic survival curves If further experiments prove the significance of those differences, a possible explanation may be a differential oxygen enhancement of the radiation damage that concerns retardation of the growth and the damage that concerns clonogenic capacity In order to test this possibility in current experiments the differences between the colony size distribution of oxically and anoxically irradiated clonogenic cell populations are being studied

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SUMMARY

An investigation was performed with cultures of the ELD ascites tumour to obtain information on the multiplication of cells irradiated with a single or a split dose of radiation in the presence or absence of oxygen. Oxygen enhanced the radiation damage concerned with clonogenic capacity to a greater extent than the damage concerned with growth retardation. Recovery due to fractionation was observed for both types of damage. The reaction of the cell strains appears to be related to differences in their ploidy grade and the content of inherent nonprotein bound sulphydryl groups.

ZUSAMMENFASSUNG

Kulturen des ELD Ascitestumors wurden benutzt, um die Zellenvermehrung nach Bestrahlung mit einer Einzeldosis oder mit einer unterteilten Dosis mit oder ohne Sauerstoff zufuhrung zu studieren. Sauerstoff erhohte die Strahlenschadigung hinsichtlich der Klon bildenden Kapazitat in hoherem Ausmass als hinsichtlich der Wachstumshemmung. Erholung als Folge von Fraktionierung war fur beide Typen von Schadigung zu beobachten. Die Reaktion verschiedener Zellkulturen scheint von Unterschieden in ihrem Ploidygrade und deren Gehalt an nicht proteingebundenen Sulphydrilgruppen abzuhangen.

RÉSUMÉ

L'auteur a fait des recherches sur des cultures de tumeur d'ascite ELD pour étudier la multiplication des cellules irradiees par une dose unique de radiation ou par une dose fractionnee en la presence et en l'absence d'oxygene. Les radio-lesions qui ont un effet sur l'aptitude clonogenique sont plus augmentees par l'oxygenation que les lesions qui interviennent dans le retard de croissance de la tumeur. Dans ces deux types de lesions on a observe une restauration due au fractionnement. La réaction des souches de cellules parait liee a des differences dans leur degre de ploidie et dans la teneur en groupes sulfhydryle inherents non liés aux protéines.

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The observation that sensitivity differences, apparent after radiation exposure under anoxic conditions, disappear when exposure is made in the presence of oxygen, is in agreement with earlier findings obtained in different experimental systems (REVESZ et coll 1963) The possible mechanisms underlying this effect of oxygen have been discussed in a previous publication (LITTBRAND 1970)

Conclusions

The multiplication of ELD ascites tumour cells and four of its substrains were studied after exposure to single or split doses of roentgen radiation under oxic or anoxic conditions The cell population increased exponentially with a doubling time of 56 hours after exposure doses of 450 R in oxygen and 1 100 R in argon When doses of 1 000 R in oxygen and 2 500 R in argon were delivered, the cell population decreased during an initial period of 11 days after treatment and, subsequently, grew exponentially with a doubling time of 22 hours The ratio between the size of the cell populations irradiated with a single dose, and with the same dose split into two fractions, was between 1 and 2 in cases of anoxic irradiation exposures, and between 2 and 20 in cases of oxic exposures After oxic irradiation, the growth of the different cell strains was inhibited to a similar extent On the other hand, after anoxic irradiation, the cell strains *presented characteristic differences in their reaction to the growth inhibitory effect* of radiation

The growth curves were analysed in view of the clonogenic survival of the cells after comparable exposure doses This analysis indicates that a great proportion of clonogenic survivors have a reduced growth rate, in addition to the *clonogenic capacity recovery during the split dose interval concerns* the growth rate and possibly also division delay The capacity of lethally injured cells for a limited number of divisions is improved by split dose treatment, repair of radiation *damage leading to recovery is, to a great extent, dependent upon* the presence of oxygen during irradiation

The particular *reaction of different cell strains can be related to the differences* in their ploidy grade and the content of inherent, nonprotein bound sulphydryl groups

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LONG TERM CYTOGENETIC AND CLINICAL CONTROL OF A CHILD FOLLOWING INTRAUTERINE IRRADIATION

by

MARIA KUČEROVÁ

Morphologic and cytogenetic lesions of the foetus are presumed to occur in irradiated pregnant women especially with radiation in the therapeutic range Experimental studies in lower mammals (HICKS 1953, SOUKUP et coll 1965) and the examination of irradiated human foetuses (HOBBS 1950, MILLER 1956 DRISCOLL et coll 1963 KLAUBER 1965 DEKABAN 1968) confirm this presumption from the morphologic point of view but relatively few chromosome studies in irradiated human foetuses have so far been performed SATO (1966) MACEK et coll (1967) analysed the chromosomes from fibroblasts of dead foetuses in long term cultures Chromosome aberrations in lymphocytes from living subjects after intrauterine irradiation with a diagnostic dose were reported by LEJEUNE et coll (1964) and with a therapeutic dose by KUČEROVÁ (1967a) In the last mentioned paper a child surviving after irradiation in the second half of pregnancy was described This child has now been systematically observed for 4 years, and the findings are summarized in the present paper

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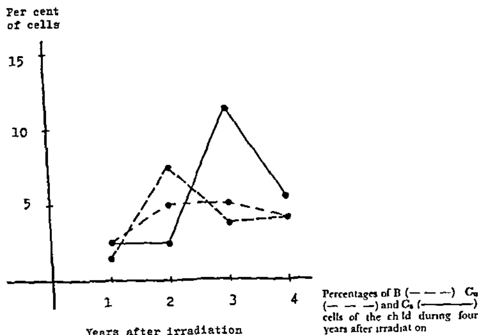
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Percentages of B (— — —) C_u (— — —) and C_s (———) cells of the child during four years after irradiation

In the diagram accompanying this paper the cells have been classified according to the Edinburgh group of authors (BUCKTON et coll 1962) as B C and C_u This classification has been used by many authors the present results can thus be compared with those of others

Four examinations in all were performed under standard conditions in the child (in December 1965 1966 1967 and 1968) In 1965 and 1966 peripheral blood from the mother was also examined

Results

Detailed cytogenetic analysis indicated a significant increase of mitoses with chromosome aberrations in all blood samples from child and mother The tabulated results (Table 1) disclosed that the percentage of aberrant mitoses in the child s blood increased with post irradiation from 6 6 % in the first year to over 15 % in the second year and up to 20 % in the third year of life The percentage fell slightly to 13 6 in the fourth year It is still not certain whether the percentage will further decrease or not A sharp increase in the percentage of aberrations

At the same time and for two years chromosomes of the peripheral blood of the mother were analysed It was of interest to find which types of aberrations occurred in the child and the mother, and the time course of these changes

Case report

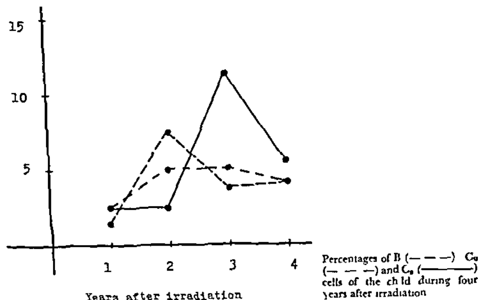
The father was 39 at the time of the child s birth and in good health Two elder sisters (one a step sister) were normal in body and mental development Carcinoma of the cervix uteri was discovered in the mother during the third pregnancy when she was 36 years old She was therefore given roentgen irradiation in the course of the 5th and 6th months of pregnancy The radiation factors were 180 kV, 10 mA, filter 1 mm Cu Four fields all 10 cm × 15 cm were exposed to radiation anterior left anterior right posterior left and posterior right with 27 6 R/min individual exposures 270 R total surface exposures of the individual fields 3 580 R The total radiation dose was divided into 26 daily radiations The dosage received by the child is not known

Hysterectomy with Caesarean section was performed in the 8th month of pregnancy The mother died of generalized carcinoma 2 years after delivery The birth weight of the child was 1 960 g and its length 44 cm No congenital malformations were observed The child sat at 9 months stood at 12 months walked at 21 months and began to talk at 36 months At the age of 1 year our patient had febrile convulsions later followed by epileptic seizures of the type major epilepsy

At the time of this report the child was 4 1/2 years old with evidence of microcephaly Her height was 95 against 103 9 cm weight 11 5 against 16 5 kg the head circumference 46 against 51 cm and that of the breast 49 against 55 cm The mental development of the child corresponded to that of a 2 year old and of a low grade imbecile She was passive and apathic Comprehension and articulation of speech were poor Neurologic examination suggested slight cerebellar changes and EEG has been abnormal with manifestations of asymmetry Slight anaemia and leucocytosis have been repeatedly observed

Methods of investigation A standard cytogenetic method of short term peripheral blood cultures, a modification of Moorhead s method (MOORHEAD et coll 1960), was employed The cultures were grown in Epl medium (USOL-Institute of Sera and Vaccines, Praha) and calf serum 25 % for an average of 64 hours, and stained with Giemsa All mitoses were photographed, cut and analysed and only those treated in this manner are described in the results (to avoid monotrisomy being overlooked)

In the classification of cells, the mitoses are grouped as hypomodal modal, hypermodal and polyploid, according to the chromosome number, and as normal and aberrant mitoses according to structural changes Aberrant mitoses are divided into simple (resulting from simple breaks) and complicated (arising from multiple breaks and reunions) and monotrisomy (cells with chromosomes of normal shape that cannot however be correctly divided into groups of the Denver nomenclature) This has been described in detail in a previous paper (KUČEROVA 1967b)



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Table 1

Chromosome analysis of peripheral leukocytes of child and mothe after therapeutic irradiation and in normal controls

Time after irradiation	Number of mitoses				Aberrant mitoses			Types of aberrations number/per cent			Per cent polyploid cells
	Number of chromosomes			Total	Number	Per cent	Total	S	C	MT	
	<46	=46	>46								
Child											
One year	19	173	4	196	13	6.6	13	3/42	6/46	4/30	11.2
Two years	3	36	1	40	6	15.0	7	4/57	2/29	1/14	—
Three years	21	51	8	80	16	20.0	22	7/32	4/18	11/50	1.2
Four years	23	49	1	73	10	13.6	13	4/31	5/58	4/31	2.6
Mother											
One year	13	67	3	83	4	6.0	5	1/20	2/40	2/40	5.6
Two years	16	41	2	59	8	13.5	11	7/63	2/18	2/18	1.6
Controls	95	577	53	725	7	0.9	7	3/43	—	4/57	0.6

was also noticeable in the mother, who had 6 % of aberrations in the first post irradiation year and 13.5 % of aberrant mitoses in the second year

The percentage of polyploids, which was high in the mother and child in the first year, fluctuated in the following years around the usual percentage in the controls

After the classification of the observed aberrations into three basic categories (*simple, complicated and monotrisomic*), certain fluctuations were noted during the following four years but the direction of these changes was not uniform The most recent results (i e 4 years after irradiation) indicate that all three categories of aberrations were more or less uniformly represented

A still more detailed analysis of aberrant mitoses i e the study of individual aberrations but not of groups of aberrant mitoses (see Table 2) revealed a gradual decline in the incidence of dicentric chromosomes (from 2.5 % to zero)

Table 2

Incidence of individual aberrations at different intervals after irradiation

Time after irradiation	B	B per cent	F	AC	DC	DC per cent	RC	TR	M	MT	MT per cent	Number of all aberrations	Number of all analysed mitoses / Number of abnormal mitoses
Child													
One year	3	1 5			5	2 5		1		4	2	13	190/13
Two years	3	7 5		1	1	2 5		1		1	2 5	7	40/6
Three years	4	5 0		3	1	1 2	1		2	11	13 5	22	80/16
Four years	3	4 1	1	3				1	1	4	5 4	13	73/10
Mother													
One year	2	2 4			2	2 4				1	1 2	5	83/5
Two years	3	5 0		4	1	1 7		1		2	3 5	11	59/8
Control	3	0 4								4	0 5	7	775/7

B = break F = fragment AC = acentric chromosome DC = dicentric chromosome RC = ring chromosome TR = translocation M = marker MT = monotrisomy

and an increase in the monotrisomy (from 2 % up to 5 4 %) Both changes can be anticipated because the dicentric chromosomes are considered to be an unstable type of aberration and monotrisomy a stable one The increase in the number of breaks (from 1 5 to 4 1 %) is interesting but difficult to explain The possibility cannot be excluded that this could be partly due to the technical procedure used which hardly affects the incidence of the other aberrations however After classifying the aberrant mitoses observed in this case as B C and C cells unexpected results were obtained The number of C cells involving aberrations regarded as unstable, usually declined following a rapid increase after irradiation In our material the level of these cells was practically

unchanged during the 4-year observation period and remained slightly elevated, with small fluctuations

The C_s cells, involving aberrations regarded as stable usually slightly increase in number after irradiation, then slowly decline and remain at a somewhat increased level for several years We found that their numbers fluctuated significantly, were relatively stable and increased slightly between the first and second years, increased much between the second and third years, and then decreased rapidly between the third and fourth years to a level higher than in the second and third years

Cells B, not included in the post irradiation changes by the Edinburgh group (they may be artificial in origin and their number ought to be relatively stable), exhibited *significant fluctuations in our studies* It is interesting that the number of B cells increased as the number of mitoses in the culture decreased As the same blood volume (10 ml) was always used, and all suitable mitoses were analysed, an explanation may be that the less readily the cells multiplied in the culture, the less resistant were their chromosomes against mechanical damage

The results of chromosome analyses in the mother and child could be compared only in the first and second years after irradiation The percentage of aberrant mitoses and the proportion of different types of aberrations did not differ substantially during the first and second investigations Differences could be found in following the percentage of individual aberrations and polyploidy The percentage of dicentric chromosomes in the mother decreased (from 2 4 to 1 7 %) between the first and second year and in the child between the third and fourth year Monotrisomy increased more significantly in the mother than in the child, from 1 2 to 3 5 % in the mother and from 2 to 2 5 % in the child The percentage of polyploidy, which was significantly increased in both the mother and child in the first year after irradiation (5 6 % in the mother and 11 2 % in the child), was in the child double that observed in the mother

Discussion

The results of the cytogenetic investigation revealed an unexpected increase in the proportion of aberrant mitoses during the first three years following irradiation and are at variance with our previous findings in adult females irradiated with a roentgen therapeutic dose (KUČEROVA, in press) and also with findings of other authors (*BUCKTON et coll* 1962, BENDER & GOOCH 1963, MILLARD 1965, BUCKTON et coll 1967), who have reported that the total number of chromosome aberrations in peripheral blood of irradiated subjects always decreases with a prolonged time interval after irradiation

The possibility is not excluded that the exceptional circumstances under which

the chromosome aberrations originated in the present case that is, the period of intrauterine life, could explain the unusual increase in the aberrations This increase was however al o noted in the mother between the first and second year following irradiation The progressive generalization of the malignancy might have had some bearing

Unfortunately there are still too many gaps in our information on the behaviour of lymphocytes with chromosome aberrations of all types in the human body The mechanism controlling their level is quite unknown The variations in the number of cells with aberrations considered as stable and those with aberrations regarded as unstable cannot as yet be satisfactorily explained

The approach of other authors who analysed the chromosomes in foetuses after intrauterine irradiation differed somewhat from that used in our case The radiation effects in the initial months of pregnancy on embryonic cells in long term tissue cultures were described by SATO (1966) and MACEK et coll (1967) LEJEUNE (1964) reported chromosomal changes in the peripheral blood and skin of a child irradiated with a diagnostic dose in the first half of the first month of pregnancy The child exhibited some malformation as well as mental retardation and certain chromosome aberrations (surplus chromosomes) According to DEKABAN (1968) it is not certain whether during the first two weeks of pregnancy the foetus can be damaged by irradiation

The clinical lesions in the present case correspond rather closely to lesions produced by experimental irradiation in young mice and rats (HICKS 1953, HULSE 1964) Those irradiated in the second half of gestation presented evidence of retardation in growth and general hypoplasia diminished volume of the brain decreased thickness of the cortex cerebri and changes in the EEG as well as disturbances in locomotor coordination Models of oligophrenia could be experimentally induced HICK reports in addition to lesions of the cortex cerebri changes in the cerebellum in young rats irradiated in the last trimester of gestation

All the above disturbances including slight cerebellar lesions were observed in the child described in this study The basic disturbances such as microcephaly as well as growth and psychomotor retardation fully agree with clinical findings in other children after intrauterine irradiation with therapeutic doses as reported by DEKABAN (1968) They also concur with those in children after intrauterine irradiation in Hiroshima as given by MILLER (1956) and PLUMER (1952) However epileptic seizures have not yet been reported in the cases described in the literature The clinical signs observed in the child examined correspond to irradiation performed between the 16th and 20th weeks of pregnancy according to DEKABAN's table though the patient was actually irradiated between the 20th to the 24th weeks when to cite DEKABAN, no anomalies should arise It seems

that the time limits of the radiation effects have not been determined accurately enough, they may alter to a certain extent, or overlap. The situation may nevertheless be affected by varying individual influences.

HOBBS (1950) and KLAUBER (1965) described cases of intrauterine irradiation of the child in the second half of pregnancy entirely free from any clinical abnormality. Presumably irradiation was performed after the critical period described by DEKABAN.

Acknowledgement

The technical assistance of Miss Olga Tilschová is gratefully acknowledged.

SUMMARY

The results of a 4-year cytogenetic and clinical control of a child subjected in the fifth and sixth months of pregnancy to a therapeutic roentgen dose for carcinoma of the cervix uteri in the mother are reported. A detailed analysis of chromosome aberrations produced some atypical findings which are discussed. The mother also underwent cytogenetic investigations.

ZUSAMMENFASSUNG

Die Resultate einer vierjährigen cytogenetischen und klinischen Kontrolle eines Kindes werden vorgelegt. Die Mutter wurde wegen eines Cervixcarcinoms während den fünften und sechsten Monaten der Schwangerschaft mit einer therapeutischen Strahlendosis behandelt. Eine detaillierte Analyse der Chromosomenaberrationen führte zu atypischen Befunden, die diskutiert werden. Die Mutter wurde auch cytogenetisch untersucht.

RÉSUMÉ

L'auteur présente le résultat de l'étude cytogénétique et clinique poursuivi pendant quatre ans sur un enfant soumis au cours des cinquième et sixième mois de la grossesse à une dose thérapeutique de rayons de roentgen pour un cancer du col utérin de sa mère. L'analyse détaillée des aberrations chromosomiques donnait quelques résultats atypiques qui sont examinés. La mère était soumise elle aussi à des examens cytogénétiques.

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RÉSUMÉ

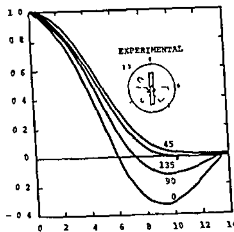
L'auteur présente le résultat de l'étude cytogénétique et clinique poursuivi pendant quatre ans sur un enfant soumis au cours des cinquième et sixième mois de la grossesse à une dose thérapeutique de rayons de roentgen pour un cancer du col utérin de sa mère L'analyse détaillée des aberrations chromosomiques donnait quelques résultats atypiques qui sont examinés La mère était soumise elle aussi à des examens cytogénétiques

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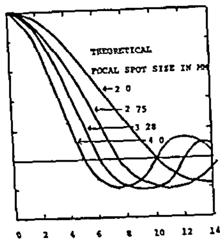


Image f eq ency — cycles per mm

a b

Fig 1 a) MTF curves of a typical roentgen ray focal spot measured experimentally along the central axis in four different directions on a plane normal to the direction of the incident beam parallel to the projected width of the focal spot (marked as 0) 45 to it 90 to it and 135 to it The focus-object distance was 100 cm and the object film distance was 5 cm. b) Theoretical MTF curves of a square fo al spot of different sizes at a focus-object distance of 100 cm and object film distance of 5 cm

various reasons it is important to be able to measure the MTF of roentgen ray focal spots experimentally Methods of making these measurements have been described by KANAMORI (1965) DOI et coll (1965) and by GOPALA RAO & BATES (1968) Using these methods it is possible to generate for each focal spot a family of MTF curves at various positions distances and angulations

Although the above mentioned method of describing the resolution characteristics of a roentgen ray focal spot is extremely rigorous from a physicist s point of view a practising radiologist interested in the intercomparison of several different roentgen tubes will find the use of families of MTF curves rather impractical From his point of view, a direct comparison of the linear dimensions of the focal spots is much simpler Yet as demonstrated by GOPALA RAO & BATES (1968) resolution cannot be judged adequately from the nominal focal spot size as quoted by the manufacturers This difficulty can be overcome if one calculates the effective dimensions of a focal spot from a knowledge of the experimentally measured modulation transfer functions

In Fig 1a the modulation transfer functions of a typical roentgen ray focal

FROM THE DEPARTMENT OF RADIOLOGY, THE JOHNS HOPKINS MEDICAL INSTITUTIONS, BALTIMORE, MARYLAND, U S A

EFFECTIVE DIMENSIONS OF ROENTGEN TUBE FOCAL SPOTS BASED ON MEASUREMENT OF THE MODULATION TRANSFER FUNCTION

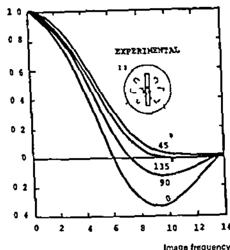
by

GOPALA U V RAO and LLOYD M BATES

In recent years, the modulation transfer function (MTF) has become a widely accepted concept for the specification of the resolution capabilities of roentgen imaging systems One of the important causes of loss of resolution in these systems is the finite size and shape of the focal spot Several years ago, MORGAN (1962) derived a theoretical expression for the modulation transfer function of a square focal spot with uniform emission

The MTF of a focal spot can be calculated from theory alone, only if it can be assumed that the focal spot is of a well defined geometric shape, such as a square, a rectangle or two parallel bars However roentgen ray focal spots in general are not composed of such perfect geometric shapes nor do they have uniform emission Furthermore, since the roentgen ray focal spots are generally designed on the line focus principle, the size of the projected focal spot varies with the angle of projection Hence radiographic resolution or the MTF also depends on the position and direction of interest in a plane normal to the central axis of the beam This effect is further accentuated by the fact that the shape of the focal spot also varies with the angle of projection For these

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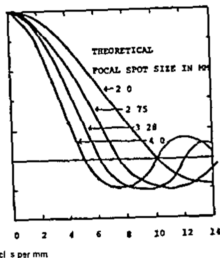


Fig 1 a) MTF curves of a typical roentgen ray focal spot measured experimentally along the central axis in four different directions on a plane normal to the direction of the incident beam parallel to the projected width of the focal spot (marked as 0) 45 to it 90 to it and 135 to it The focus-object distance was 100 cm and the object film distance was 5 cm. b) Theo etical MTF curves of a square fo al spot of different sizes at a focus-object distance of 100 cm and object film distance of 5 cm

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In Fig 1a the modulation transfer functions of a typical roentgen ray focal

Table 1

Effective dimensions of a typical focal spot

λ	0°		45°		90°		135°	
	Freq at which the MTF is λ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is λ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is λ (cycles/mm)	Eff focal spot size in mm	Freq at which the MTF is λ (cycles/mm)	Eff focal spot size in mm
0.8	2.2	3.27	3.0	2.40	2.6	2.77	2.7	2.67
0.6	3.4	3.12	4.4	2.41	4.0	2.65	4.1	2.59
0.4	4.3	3.16	5.6	2.43	5.0	2.72	5.3	2.57
0.2	5.2	3.19	7.1	2.34	6.0	2.77	6.7	2.48
0.0	6.1	3.28	—	—	7.3	2.74	—	—
	Mean focal spot size	3.20		2.40		2.73		2.58

spot, measured along the central axis in four different directions on a plane normal to the direction of the incident beam are recorded parallel to the projected width of the focal spot (marked as 0° in the figure), 45° to it, 90° to it and 135° to it. In Fig. 1 b are shown the theoretical MTF curves for various sizes of focal spots, calculated using the standard theoretical formula (MORGAN 1962) for the MTF of a square roentgen ray focal spot.

The modulation transfer function $M(f_i)$ of a square focal spot of projected width a is given by the expression

$$M(f_i) = \frac{\sin(\pi f_i a d_2/d_1)}{(\pi f_i a d_2/d_1)}$$

where f_i is the spatial frequency in cycles per millimeter in the image plane of a test object with sinusoidal transmission placed at a distance of d_1 from the focal spot and a distance d_2 from the film.

Looking at the curve for zero degrees in in Fig. 1 a, one observes that the MTF passes through zero at an image frequency of 6.1 cycles/mm. Looking at the theoretical curves on the right hand side, it is seen that the MTF of a square focal spot of 3.28 mm also passes through zero at the same spatial frequency. It follows therefore, that as far as the zero degree position is concerned, the roentgen tube in question may be said to have an effective focal spot size of 3.28 mm.

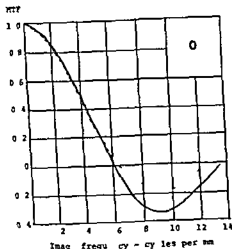


Fig 2 Diagram showing the agreement be tween the experimental MTF curve (solid) and that calculated theoretically using the concept of a mean effecti e size (dotted) The curves shown refer to the 0 position of fig 1

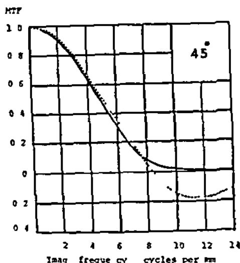


Fig 3 D agram showing the agreement be tween the experimental MTF curve (solid) and that calculated theoretically using the concept of mean effective size (dotted) The curves shown refer to the 45 position in fig 1

The MTF of a theoretical square focal spot of size a passes through zero at an image frequency f given by the equation $f_i = d_1/ad$ (see formula p 364) Therefore in general if the MTF obtained experimentally at a focus to object distance d_1 and an object to film distance d passes through zero at an image frequency $(f)_0$ an effective focal spot size can be calculated using the equation

$$\text{eff size} = \frac{1}{(f_i)_0} \frac{d_1}{d_2} \qquad (1)$$

In a similar manner it is possible to compare the frequencies at which the experimental and theoretical MTF curves have similar values other than zero for example say 0 2 According to the theoretical equation for a square focal spot (see formula p 364) the MTF becomes equal to 0 2 when $f_i = 0\,83\, d_1/ad$ This means that if an experimentally measured MTF curve has a value of 0 2 at an image frequency $(f)_{0\,2}$ an effective focal spot size can be calculated using the equation

$$\text{eff size} = \frac{0\,83}{(f_i)_{0\,2}} \frac{d_1}{d_2} \qquad (2)$$

Table 1

Effective dimensions of a typical focal spot

λ	0		45		90		135	
	Freq. at which the MTF is λ (cycles/mm)	Eff. focal spot size in mm	Freq. at which the MTF is λ (cycles/mm)	Eff. focal spot size in mm	Freq. at which the MTF is λ (cycles/mm)	Eff. focal spot size in mm	Freq. at which the MTF is λ (cycles/mm)	Eff. focal spot size in mm
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The effective dimensions of the focal spot of Fig. 1 are given in Table 1, in four directions on a plane normal to the central axis of the beam. These were calculated using equation (3) above for various values of x ranging from 0 to 0.8. The last column of the table shows the mean effective focal spot dimensions in each of these four directions. The experimental MTF curve and that calculated theoretically for the 0° position using the mean effective size, are recorded in Fig. 2. The agreement between the theoretical and the experimental curves in the case of the 45° position is apparent from Fig. 3.

The mean effective focal spot dimensions of several different roentgen tubes are presented in Table 2. In general, the effective focal spot dimensions as calculated from experimentally measured MTF data may be seen to be considerably larger than the nominal size claimed by the manufacturers. They also depend to a considerable extent on the direction of interest in a plane normal to the central axis of the incident beam.

Conclusion

Above has been presented a method of calculating the mean effective dimensions of roentgen ray focal spots on the basis of experimentally measured modulation transfer function data. The method described is not rigorous in the sense that it assumes that experimentally measured modulation transfer functions of any shape can be matched with the theoretical curves for a linear spot of appropriate dimensions. It may be argued that this assumption is not always quite valid. However, we have found that the method yields extremely satisfactory agreement betwen the experimental and the theoretical curves except in the region of spatial frequencies when the MTF becomes negative (see Fig. 2). This however is of no serious practical significance, since the region of negative MTF is important only when strictly periodic patterns are imaged. As far as the imaging of finite structures is concerned the region of negative MTF does not contribute significantly to image deterioration because the absolute values of the MTF are usually very small in this region.

SUMMARY

A method is described for the calculation of mean effective dimensions of a roentgen ray focal spot based on an experimental measurement of the modulation transfer function.

ZUSAMMENFASSUNG

Eine Methode wird beschrieben die mittlere effektive Grosse des Fokus Flecken von Rontgenstrahlen auf der Basis von experimentellen Messungen der Modulation der Transfer Funktion abzuschatzen.

Table 2

Mean effective dimensions of the focal spots of four typical roentgen ray tubes expressed in millimeters

Tube No.	Small focal spots: Nominal size according to manufacturer	Mean effective size 0	45	90	135	Large focal spots: Nominal size according to manufacturer	Mean effective size 0	45	90	135
1	0.5	1.44	1.15	0.82	1.15	1.5	2.14	1.63	1.98	1.71
2	1.0	1.65	1.41	1.54	1.41	2.0	2.69	2.57	2.72	2.61
3	1.0	1.79	1.32	1.55	1.35	2.0	3.20	2.40	2.73	2.58
4	1.0	1.68	1.40	1.51	1.41	2.0	2.9[illegible]	2.41	2.73	2.5[illegible]

In general, if an experimentally obtained MTF curve has a value x at frequency $(f_i)_x$, an effective focal spot size can be calculated using the equation

$$\text{eff. size} = \frac{\tau_x}{(f_i)_x}\,\frac{d_1}{d_2} \qquad (3)$$

x	τ_x
0	1.00
0.2	0.83
0.4	0.68
0.6	0.53
0.8	0.36

where τ_x is a constant that depends on x.

The value of τ_x for any particular value of x is simply the normalized frequency f_i/f_d (f_d being equal to d_1/ad_2) at which the MTF of a focal spot calculated from MORGAN's theoretical expression becomes equal to x. In other words, x and τ_x are related to each other by the equation

$$x = \frac{\sin(\pi\tau_x)}{(\pi\tau_x)}$$

If x is selected as zero, $\tau_x = 1.0$ and equation (3) obviously reduces to equation (1). Likewise if x is selected as 0.2, τ_x equals 0.83 and equation (3) reduces to equation (2).

The use of the generalized equation can be illustrated by a further example. Looking at Fig. 1 again, we find that the MTF in the case of the 135° position has a value of 0.2 at an image frequency of 6.7 cycles/mm. Substituting $(f_i)_x = 6.7$ and $\tau_x = 0.83$ in equation (3), the effective focal spot size in this case can be seen to be $\frac{0.83}{6.7} \times \frac{100}{5}$ or 2.48 mm.

FROM THE DEPARTMENT OF PATHOLOGY II, UNIVERSITY OF UMEÅ, SWEDEN

MODIFYING EFFECTS OF CYSTEAMINE ON EXPERIMENTAL RADIATION LESIONS OF THE BRAIN

by

OVE HASSLER

Compounds that afford protection against the effects of ionizing radiation have become of great biological and military importance in recent years. It has, for instance, been estimated that if the compound at present most commonly used for protection (cysteamine) could be administered effectively in connection with a total nuclear attack on the USA, 12 million lives would be saved (NELSON & AKERFELDT 1967), and even more effective protective compounds are under investigation.

Compounds administered to protect against radiation are absorbed in varying degrees by the various tissues (NELSON & ULLBERG 1960). It can therefore be expected that radioprotective agents affect the different tissue components to different extents so that radiation damage can be modified selectively by choosing between the various protective agents. The objective of the present work has been to study whether and how a radiation lesion of the brain may be modified by cysteamine. Knowledge of such modifications may be of use in radiation treatment of malignant tumours and other disorders and should also increase the understanding of the pathomechanisms of a radiation lesion.

This work was supported by Grant No. B69-12X-561-05 from the Swedish Medical Research Council. Submitted for publication 23 September 1969.

RÉSUMÉ

Les auteurs decrivent une méthode pour calculer les dimensions moyennes efficaces de la tache focale d un tube a rayon de roentgen méthode basée sur la mesure experimentale de la fonction de transfer de modulation

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Table

Distribution of various types of radiation lesions in fields irradiated with and without cysteamine. In group I the dose administered was the same to both fields (2 300 rad 1 cm below the skin in the brain) while in group II the dose to the cysteamine field was 50% higher (3 450 rad). A — animals with no lesions. B = animals with lesion in the control field only. C = animals with lesion in the cysteamine field only. D = animals with lesions in both fields

	Group I (ten rabbits)				Group II (ten rabbits)			
	A	B	C	D	A	B	C	D
Inflammation of skin	2	4	0	4	1	0	2	7
Skin erosions	5	3	0	2	2	0	3	5
Complete epilation	7	3	0	0	6	0	2	2
Incomplete or complete epilation	1	7	0	2	0	0	0	10
Incomplete hair covering when killed	1	2	0	7	0	0	1	9
Hyperkeratosis	3	5	0	2	1	0	3	6
Skin atrophy	1	5	0	4	1	0	0	9
Telangiectases in white matter	4	4	0	2	2	0	2	6
Marked telangiectases in white matter	8	2	0	0	7	0	2	1
Telangiectases in grey substance	8	2	0	0	7	0	2	1
Meningeal fibrosis	10	0	0	0	7	0	3	0
Meningeal inflammation	8	2	0	0	4	1	3	2
Fibrin in ventricular walls	9	1	0	0	5	0	3	2
Fibrosis of choroid plexus	10	0	0	0	6	0	3	1
Inflammation of the choroid plexus	10	0	0	0	8	0	2	0
Haemorrhage	5	4	1	0	4	1	2	3
Large haemorrhage	10	0	0	0	9	0	1	0
Endothelial proliferation and swelling	6	3	1	0	5	0	2	3
Fibrinoid necrosis of arterial wall	8	2	0	0	6	0	3	1
Hyalinosis of arterial wall	6	4	0	0	5	0	2	3
Perivascular inflammation	6	3	1	0	5	1	2	2
Plasmatic impregnation	8	2	0	0	8	0	2	0
Fat granule cell	6	4	0	0	5	0	3	2
Fibrous scar in the cerebral parenchyma	9	1	0	0	6	0	2	2
Calcifications	6	4	0	0	5	0	3	2
Amyloid like material	9	1	0	0	6	0	2	2
Necrosis or cysts in white matter	6	4	0	0	5	0	3	2
Large necrosis or cysts in white matter	8	2	0	0	7	0	1	2
Necrosis or cysts in grey substance	8	2	0	0	7	0	1	2
Loss of normal architecture	3	5	0	2	1	0	4	5
Destruction of myelin sheaths	0	5	1	4	0	0	3	7
Glial foci	6	4	0	0	3	0	4	3
Astroglial hypertrophy	10	0	0	0	7	1	1	1
Atypical microglia	9	1	0	0	8	0	2	0
Fibrillary gliosis	9	1	0	0	8	1	1	0
Enlarged oligodendroglia	10	0	0	0	8	0	2	0
PAS positive granules in oligodendroglia	8	2	0	0	4	0	4	2
Spongiosus in ph l my l t s	10	0	0	0	9	0	0	1

Materials and Methods

Autoradiographic study in mice with ^3H thymidine. Twelve adult male white mice of an inbred strain (N. M. R. I., Bethesda, supplied by Anticimex, Norrviken, Sweden), belonging to two litters and weighing 18 to 22 g at the start of the experiment, were employed. One (control) cerebral hemisphere was irradiated by roentgen rays as in a previous work (HASSLER 1966). Within 5 minutes after this irradiation 5 mg of cysteamine HCl (Th. Schuchardt GmbH, Munich, West Germany) were dissolved in sterile water, neutralized with dilute NaOH to pH7, and injected intraperitoneally into each mouse a few minutes before a second irradiation which was performed in the same way as the first one but symmetrically on the opposite hemisphere. Thus, one (control) hemisphere was irradiated without and the other with administration of cysteamine. The doses were the same in both hemispheres (2 370 ± 237 rad 4 mm below the skin). All the animals were killed 7 days after irradiation.

The autoradiographic study with ^3H thymidine (supplied by the Radiochemical Centre, Amersham, England), the histologic investigation, and the counting of labelled cells, were performed in the same way as in a previous work (HASSLER 1966).

Microangiographic and histologic studies in rabbits. Twenty male rabbits (Swedish Landrace) weighing 1.5 to 2.0 kg at the time of irradiation, were used. They were divided into two groups according to the doses of radiation administered. In all the animals, one (control) cerebral hemisphere was irradiated by ^{60}Co as in a previous study (HASSLER & MOVIN 1966). Within 5 minutes following irradiation, a solution of cysteamine (200 mg/kg body weight), prepared freshly as above, was injected intravenously, and the opposite hemisphere was irradiated a few minutes later in a similar way.

In the first ten animals, group I, the dose to the opposite hemisphere was the same as in the control hemisphere (2 300 ± 230 rad in the brain 1 cm below the skin). In the remaining ten animals, group II, the dose to the opposite hemisphere was 50 % higher (3 450 ± 345 rad 1 cm below the skin), in order to obtain a similar effect in both hemispheres.

The animals were examined for skin reactions (see Table) every third day for 10 to 40 days following irradiation, thereafter the examinations were performed only once a week. When complete epilation occurred within an area of at least 0.75 cm², the irradiation field was tabulated under "complete epilation".

All the animals, except two, were killed 360 days after irradiation. The two animals (both belonging to group II) became ill and lost appetite and weight, when their condition was judged to be hopeless, they were killed (274 and 309 days after irradiation respectively). Microangiography of all the twenty animals



Fig 2 Histologic sections through small representative parts of the sections shown in fig 1a and 1b respectively ×128 The histologic changes correspond well with the angiographic appearances

All the slides were assessed as unknown (a blind study) when the histologic changes (cf Table) were graded If the soft meninges were so fibrosed and thickened that they were more than 100 μ in the sections, this was recorded in the table When more than eight inflammatory cells occurred in the meninges of one section this was registered as meningeal inflammation Fibrosis of the plexus choroideus was also recorded when it occupied an area exceeding 0 5 mm in one section Inflammation of the choroid plexus was registered when more than eight inflammatory cells occurred in one section Large haemorrhage indicates bleeding exceeding 1 mm^3 Hyalinosis of the arterial wall was diagnosed only when all muscle cells had disappeared in an artery greater than 100 μ in diameter Perivascular inflammation was registered when at least ten inflammatory cells were seen around the vessels in a section Fibrous scars in the cerebral parenchyma had to occupy at least 0 2 mm in one section to be recorded Large necrosis or cyst was diagnosed when the total volume exceeded 1 mm^3 The normal architecture had to be lost within 4 mm in one section

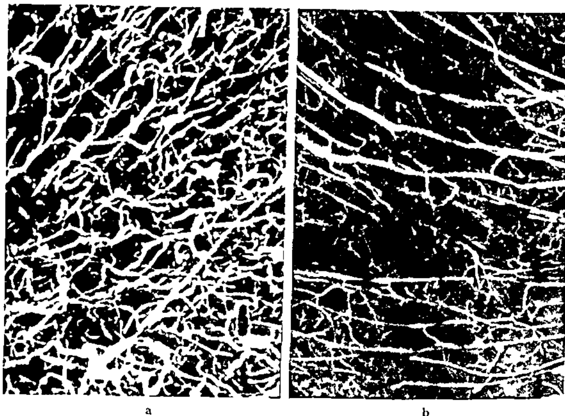


Fig 1 Microangiograms from cross sections through the dorsal superior part of the brain stem of rabbit group I a) Left side (control) irradiated with ^{60}Co γ rays 2 300 rad ×43 b) Right side Cysteamine was injected intravenously immediately before the same dose of ^{60}Co γ rays was given ×43 The hemisphere protected by cysteamine had fewer angiographic changes

was performed as in a preceding study (HASSLER & MOVIN 1966) Teleangiectases also were measured as in that study, if longer than 1 mm, they were classified as marked teleangiectases

Five slices in the frontal plane from each cerebral hemisphere were embedded in paraffin, these were cut from the same parts of the brain in all the animals and so that as many parts of the brain as possible were represented Eight sections from each slice were prepared and stained with van Gieson s stain, haematoxylin eosin, Nissl's stain, PAS and McMahon s myelin stain as well as by Ranke s Victoria blue for astrocytes (SILVERTON & ANDERSON 1961), Palmgren s silver technique for axons (PALMGREN 1948), and Ladewig s modification of the Mallory stain (ROULET 1948) When precipitations were seen, sections stained with von Kossa s stain and Turnbull blue (ROMEIS 1948) were also prepared and when amyloid like material was encountered, the alkaline Congo red procedure (PUCHTLER, SWEAT & LEVINE 1962) was applied

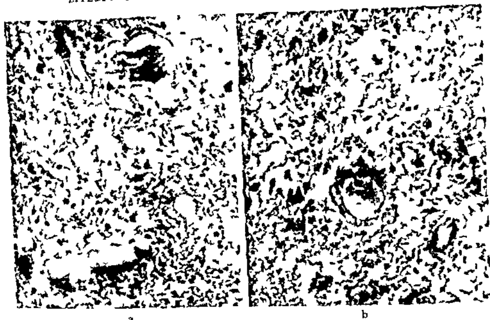


Fig 4 Histologic sections through representative parts of the sections shown in fig 3a and 3b respectively ×118 The angiographic and histologic changes were equally marked on both sides

of variations in thickness of the sections When the number of labelled cells of various types on the side irradiated with cysteamine in each section was calculated as a percentage of the corresponding number from the control irradiated side these sources of error were eliminated It could then be observed that a significant difference ($p < 0\,01$) existed in the largest group (small and dark cells)

Microangiographic and histologic studies in the rabbit All the animals, with the exception of the two killed earlier than one year of observation gained in weight from 2 9 to 4 3 kg Several animals, especially in group II had various neurologic signs (anisocoria vestibulo-cerebellar disturbances wry neck) abscesses tumours or marked hydrocephalus were never noted

The distribution of the skin reactions given in the Table reveals marked inter individual variations although much smaller variations occurred in the individuals The cysteamine side in group I was always less changed than the control side while the opposite occurred in group II The hair that grew after epilation in the pigmented rabbits was usually whitish grey

The results of microangiography (telangiectases in the Table) were in good

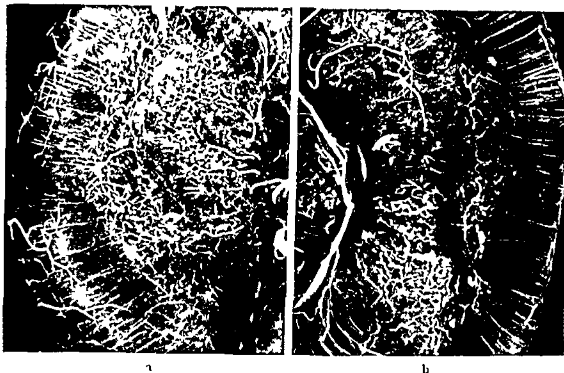


Fig. 3. Microangiograms from 2 mm thick coronal slices through the posterior part of the brain of rabbit group II. ×8. a) Left side (control) irradiated with ^{60}Co γ-rays (2 300 rad). b) Right side which after injection of cysteamine received 3 450 rad ^{60}Co radiation. Both sides had marked vascular changes with teleangiectases mainly in the white matter.

in order to be included. Glial foci had to comprise at least five cells in close contact with each other to be counted.

Results

Autoradiographic study in mice. No animal died spontaneously or presented any changes during the week between irradiation and killing. No marked abnormalities could be discovered in the histologic sections except for some pyknotic cells in the radiosensitive subependymal layer bilaterally. Small and dark cells, interpreted as precursors to oligodendroglia cells, occurred in the hemisphere irradiated without cysteamine, numbering a total of 18.6 ± 3.1 labelled cells in the counted area of each section, while in the hemisphere irradiated with cysteamine they numbered 13.7 ± 2.8. The corresponding figures for the subependymal cells were 3.8 ± 1.0 and 5.1 ± 1.4, respectively. The figures for the microglia cells, the endothelial cells and the unclassified cells, which were all grouped together, were 3.5 ± 1.6 and 5.0 ± 1.8, respectively. The figures for the standard deviation of the values were comparatively high because

in whole body autoradiography is low) The second explanation would seem to be supported by the results of the present autoradiographic studies which indicated that the cells incorporating ^3H thymidine are protected by the cysteamine so that more normal values are obtained (cf HASSLER 1966) when cysteamine is administered at the same time as the irradiation

The present investigation indicates that after the administration of cysteamine the dose of radiation cannot be increased by as much as 50 % if the same effect on the brain and skin as without cysteamine is desired This is not quite in accordance with observations regarding changes in mean lethal doses after the administration of cysteamine because the dose could then be further raised i e by 86 % (BACQ 1954) or by 67 % (NELSON 1965) The difference could in part be due to the unavoidable measuring inexactitude in all such work and to differences in the doses and animal species Another explanation could be that the mechanisms determining the lethality in total body irradiation are more affected by cysteamine than are the skin vessels and the brain

When the present work was planned it was believed that irradiation with cysteamine might produce a lesion different from those ordinarily associated with radiation This was not proved however The unavoidable individual variations are great in all irradiation work and cause as large variations as cysteamine Within the individuals the variations are much smaller It therefore would seem to be advantageous to have the field irradiated with cysteamine and the control field without in one and the same animal Inter individual variations can then be avoided

SUMMARY

The modifying effect of cysteamine on experimental radiation lesions in the brain was studied in 12 mice and 20 rabbits One cerebral hemisphere was irradiated before and the other immediately after the administration of cysteamine The radioprotective effect of cysteamine was almost equally great in skin vessels and the brain parenchyma but less than that measured in the mean lethal dose in total body irradiation

ZUSAMMENFASSUNG

Der modifizierte Effekt von Cysteamine auf experimentelle Strahlungsschaden im Gehirn wurd bei 12 Mausen und 20 Kaninchen untersucht Fine Gehirnhalfte wurde vor und die andere unmittelbar nach der Injektion von Cysteamine bestrahlt Der strahlungsschutzende Effekt von Cysteamine war ungefahr gleich gross in der Haut den Cefasswanden und dem Gehirngewebe aber geringer als der in durchschnittlich todlicher Dosis gemessene nach Totalkorperbestrahlung

agreement with the observations on the skin and with the histologic findings. The inter individual variations were much *greater than those in the individuals* themselves also as regards the telangiectases. Cystic defects in the brain parenchyma were generally closely associated in the angiograms with the telangiectases. Both the telangiectases and the cystic defects occurred mainly in the white matter, and when exceptionally large, also interfered with the grey substance. The same rabbits had marked telangiectases in the white matter and telangiectases in the grey substance. The size of the cystic defects correlated well with the size of the telangiectases.

As may be seen from the Table, the vascular changes seen in the histologic sections had a similar distribution as the changes observed in the skin and in the microangiograms. This also applies to the changes in the cerebral parenchyma. All were more marked in the white than in the grey matter. No qualitative differences could be discovered in the tissues irradiated with cysteamine. Signs of so called *spontaneous encephalomeningitis were noted in only one rabbit*. The changes were weak to moderate and because they were practically equally developed on both sides, they were presumed not to have influenced the results. The rabbit belonged to group II, had bilateral meningeal inflammation, no inflammation in the choroid plexus and no endothelial proliferation or swelling.

Discussion

It was assumed *for several reasons when the present work was started* that radiation lesions in the vessels but not in the cerebral parenchyma could be diminished by the administration of cysteamine. It was recognized that cysteamine has almost no effect on the acute radiation sickness, with cerebral symptoms and coma, following excessively high doses of total body irradiation (RUGH & CLUGSTON 1954, MAISIN & DOHERTY 1960, SORBO 1965). It was also known that ³⁵S cysteamine is incorporated to a moderate extent into the mouse skin and blood vessels and to a very small degree into the brain (NELSON & ULLBERG 1960).

The *administration of cysteamine in the present* work appeared to diminish the lesions equally in the blood vessels and the brain parenchyma. Two different explanations may be offered for this. The changes in the brain parenchyma may in some measure be secondary to those in the vessels, as has been suggested by several investigators (cf. BERG & LINDGREN 1958). Cysteamine would work mainly in the vessels, inhibiting local changes and secondarily inhibiting those in the cerebral parenchyma. The other possible explanation is that cysteamine is incorporated into strategically important parts of the brain, for example the mitotically active glia cells (although the total incorporation in the brain visible

International Commission on Radiation Units and Measurements

REPORT TO THE INTERNATIONAL EXECUTIVE COMMITTEE OF THE XIIth INTERNATIONAL CONGRESS OF RADIOLOGY TOKYO 1969

Before turning to the actual program with which the ICRU has been engaged since the XIth International Congress of Radiology it would seem appropriate to mention some of the results of the work which was reported to the International Executive Committee in 1965 and . . . ICRU efforts between 1959 and 1962 resulted in the publication of six major reports

ICRU Report 10a — Radiation quantities and units (published as NBS Handbook 84)
ICRU Report 10b — Physical aspects of irradiation (published as NBS Handbook 85)
ICRU Report 10c — Radioactivity (published as NBS Handbook 86)
ICRU Report 10d — Clinical dosimetry (published as NBS Handbook 87)
ICRU Report 10e — Radiobiological dosimetry (published as NBS Handbook 88)
ICRU Report 10f — Methods of evaluating radiological equipment and materials (published as NBS Handbook 89

Judging from the sales of these reports the work represented by them has been widely accepted in the scientific community Even today the demand for these reports continues. Further the other publications resulting from Commission activities (See Annex) have also received wide dissemination.

Substructure and mode of operation adopted in 1962

In the report submitted to the International Executive Committee in 1965 it was pointed out that the period from 1962 to 1965 was devoted principally to elaboration of the substructure and mode of operation adopted by the Commission in 1962 and to the utilization of that substructure for the initiation of new projects The period since the 1965 report has been utilized to carry forward the projects initiated with the new substructure In addition the Commission made one significant modification in the mode of operation during this time period This was the establishment in 1968 of the ICRU publications program with which the Commission began the publication of ICRU reports under its own auspices

Activities during the period 1965 to 1969

The time period from 1965 through 1969 is particularly significant because a number of ICRU reports were completed during this period In 1968 the Commission completed and published as the first reports available from the ICRU Publications Office

RÉSUMÉ

L'auteur a étudié sur 12 souris et 20 lapins l'effet modificateur de cystéamine sur les radio-lésions expérimentales du cerveau. Un hémisphère cérébral a été irradié avant et l'autre immédiatement après l'administration de cystéamine. L'effet radio-protecteur de la cystéamine a été presque aussi important au niveau de la peau, des vaisseaux et du parenchyme cérébral mais a été moindre que l'effet mesuré sur la dose léthale moyenne au cours de l'irradiation totale du corps.

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ICRU Report 11 — Radiation quantities and units

ICRU Report 12 — Certification of standardized radioactive sources

Completed in 1968 and now being prepared for publication are three additional reports formulated under the substructure adopted in 1962

ICRU Report 13 — Neutron fluence neutron spectra and kerma

ICRU Report 14 — Radiation dosimetry X rays and gamma rays with maximum photon energies between 0 6 and 50 MeV

ICRU Report 15 — Cameras for image intensifier fluorography

Nearing completion and to be considered by the Commission at its meeting in 1969 are the following reports

Specification of high activity gamma ray sources

Radiation dosimetry X rays from 5 to 150 kV

Linear energy transfer

Measurement of absorbed dose at a point in a standard phantom

Methods of arriving at absorbed dose at any point in a patient

Radiation protection instrumentation and its application

In addition to the above reports the current reporting period has seen the completion of two additional reports prepared by the authors at the reque t of the ICRU

BROWNELL G L, BERMAN M and ROBERTSON J S Nomenclature for tracer kinetics *Int J appl Radiat* 19 (1968) 249

MACINTYRE W J FEDORUK S O HARRIS C C et coll Sensitivity and resolution in radioisotope scanning *In* Proceedings of the Symposium on Medical Radioisotope Scintigraphy International Atomic Energy Agency Vienna 1969

Still in preparation in ICRU subgroups are reports on the following subjects

Measurements of low level radioactivity

Display characteristics for radioisotope scanning

Methods of as essment of dose in tracer investigations

Electron beam dosimetry

High energy and space radiation dosimetry

Methods of compensating for body shape and inhomogeneity and of beam modification for special purposes

Statement of the dose achieved

Modulation transfer function its definition and measurement

From these lists of reports it is obvious that the substructure and mode of *operation* adopted by *the Commission in 1962* involved a very substantial *effort by the Commission* to meet the expanding need for ICRU recommendations The substructure developed to meet these needs was adopted on an experimental basis to alleviate some of the problems associated with the expanded program required Previously the Commis ion s attempt to administer and review the work of each of the working groups imposed a very considerable burden on the Commission itself The need to concern itself with each detail which was inherent in such a scheme of operation when coupled with *the procedure of completing* all reports at one time subjected the Commission members to an intolerable work load if rigorous standards were to be maintained As can be seen from the list of reports completed the substructure and mode of operation adopted in 1962 has now produced results These indicate that the substructure and mode of operation while not perfect and in some ca es rather cumbersome has to a substantial extent succeeded in alleviating many of the prob lems previously experienced

L H Gray Medal

Another important action taken by the Commission during the period since the XIth Congress of Radiology was the implementation with the cooperation of the International Society of Radiology and the International Congress of Radiology of the Commission s decision to establish an ICRU medal honoring the late L H Gray former Vice Chairman of the ICRU and a recognized leader in radiation quantities and units and in radiobiology The Commission determined that the medal will be awarded for outstanding contributions in the scientific fields of interest to the ICRU It was decided that the medal may be awarded once in each four years although the scientific work on which the award of the medal is based need not have occurred during the four year period prior to the award In 1967 and 1968 the Commission gave careful consideration to the many nominations received for the first award of the medal in 1969 The Commission then determined that the first recipient of the L H Gray medal is to be Dr Louis V Spencer a physicist at the National Bureau of Standards and Professor of Physics at Ottawa University (Kansas) Dr Spencer will receive the award at the XIIth International Congress of Radiology The award to Dr Spencer is based on his long term study of the theory of charged particle penetration Dr Spencer has been invited to give a scientific lecture to the ICRU and its guests during the International Congress of Radiology

ICRU relationships with other organizations

One of the features of ICRU activity in the last few years has been the development of relationships with other organizations interested in the problems of radiation quantities units and measurements In addition to its close relationship with the ICRP and its financial relationships with the International Society of Radiology the World Health Organization and the International Atomic Energy Agency the ICRU has also developed relationships of varying intensity with several other organizations Since 1955 the ICRU has had an official relationship with the World Health Organization (WHO) whereby the ICRU is looked to for primary guidance in matters of radiation units and measurement and in turn the WHO assists in the worldwide dissemination of the Commission s recommendations In 1960 the ICRU entered into consultative status with the International Atomic Energy Agency The Commission has a formal relationship with the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) whereby ICRU observers are invited to attend UNSCEAR meetings

The ICRU and the International Organization for Standardization (ISO) informally exchange notifications of meetings and the ICRU is formally designated for liaison with two of the ISO technical committees The ICRU also corresponds and exchanges final reports with the following organizations Bureau International des Poids et Mesures Council for International Organizations of Medical Sciences Food and Agricultural Organization International Council of Scientific Unions International Electrotechnical Commission International Labor Organization International Union of Pure and Applied Physics United Nations Educational, Scientific and Cultural Organization

The Commission has found its relationship with all of these organizations fruitful and of substantial benefit to the ICRU program

Relations with these other international bodies do not affect the basic affiliation of the ICRU with the International Society of Radiology

Commission finances

Throughout most of its existence the ICRU has operated essentially on a voluntary basis with the travel and operating costs being borne by the parent organizations of the participants (only token assistance was originally available from the International Society of Radiology) Recognizing the impracticability of continuing this mode of operation on an indefinite basis operating funds were sought from various sources

Prior to 1959 the principal financial assistance for the ICRU had been provided by the Rockefeller Foundation which supplied some $11 000 to make possible various meetings. In 1960 the Rockefeller Foundation supplied an additional sum of some $4 000 making possible a meeting of the Quantities and Units Committee in 1960 In 1959 the International Society of Radiology increased its contribution to the Commission providing $3 000 for the period 1959 to 1962 For the period 1962 to 1965 this was again increased the Society providing $5 000 In March 1969 the Commission received $3 500 from the Society as the first increment of the 1965 to 1969 contribution In July 1969 an additional $4 000 was received

In 1960 and 1961 the World Health Organization made available the sum of $3 000 each year This was increased to $4 000 in 1962 and this amount has been made available annually since then It is expected that this sum will be allocated annually at least for the next several years

In connection with the Commission s Joint Studies with the ICRU the United Nations allocated the sum of $10 000 for the joint use of the two Commissions

The most substantial contribution to the work of the ICRU has come from the Ford Foundation In December 1960 the Ford Foundation made available to the Commission the sum of $37 000 per year for a period of five years This grant was to provide for such items as the travel expenses involved in attendance at meetings secretarial services and other operating expenses In 1965 the Foundation agreed to a time extension of this grant making available for the period 1966 to 1970 the unused portion of the original grant To a large extent it is because of this grant that the Commission has been able to move forward actively with its program

In 1963 the International Atomic Energy Agency allocated the sum of $6 000 per year for use by the ICRU This was increased to $9 000 in 1967 It is expected that this sum will be allocated annually at least for the next several years

From 1934 through 1964 valuable indirect contributions were made by the U S National Bureau of Standards where the Secretariat resided The Bureau provided substantial secretarial services publication services and travel costs in the amount of several thousands of dollars

The Commission wishes to express its deep appreciation to all of these organizations and to the others that have contributed so importantly to its work

Membership

In the period since the XIth International Congress of Radiology the election of two new Commission members was made necessary by the resignations of J W Boag and H F Johns Elected to fill these vacancies were J R Greening and A Tsuya

Thus the current membership consists of the following

A Allisy France R H Chamberlain U S A F P Cowan U S A J F Fowler United Kingdom F Gauwerky Germany J R Greening United Kingdom K Liden Sweden

R H Morgan USA H H Rossi USA L S Taylor USA A Tsuya Japan M Tubiana France and H O Wyckoff USA

Serving as Commission Officers until the XIIth International Congress of Radiology are L S Taylor Chairman M Tubiana Vice Chairman H O Wyckoff Secretary and W R Ney Technical Secretary

Elected at the recent meeting of the Commission were

Member Emeritus and Honorary Chairman L S Taylor

New members 1969 to 1973 A M Kellerer Germany W K Sinclair USA F W Spiers United Kingdom and A Wambersie Belgium

Continuing members 1969 to 1973 A Allisy France F P Cowan USA F Gauwerky Germany J R Greening United Kingdom K Liden Sweden R H Morgan USA H H Rossi USA A Tsuya Japan and H O Wyckoff USA

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Commission Officers H O Wyckoff Chairman A Allisy Vice Chairman K Liden Secretary and W R Ney Technical Secretary

Outlook for the future

On the basis of the ICRU s experience during the last four years the outlook for the future is bright indeed The helpful response of all of the individuals and organizations interested in the Commission s program augurs well for the future For the substantial help received from these individuals and organizations the Commission expresses its deep appreciation

The activities of the currently active ICRU subgroups are progressing well and in the near future the Commission should be in a position to publish several new reports Administratively the activities of the ICRU seem soundly based Funding appears to be the most critical problem facing the Commission especially over the next two or three years The availability of the Ford Foundation funds terminates by the end of 1970 but they will have been exhausted by about the middle of the year In the meantime the Commission is embarking upon a plan to recoup some of its expenses through its publication program This will entail raising the selling price of its reports but it is believed that this will not seriously cut down the wide dissemination of its recommendations However it is not expected that this source of income will meet the costs of operating the publications program for about two years After that the publication is expected to provide appreciable funds for ICRU operations

Thus the next two or three years will be financially critical Strenous efforts will have to be made by the Commissions its present supporters and its friends to obtain some additional funding during that period The only alternative will be to drastically curtail its efforts, even on programs that are approaching completion

Nevertheless it is evident that in spite of the funding problems the current status of the Commission s program viewed in its entirety and in the long range justifies confidence in the future

Respectfully submitted
Lauriston S Taylor

ANNEX

ICRU materials published outside of the ICRU Reports Series

Exposure of man to ionizing radiation arising from medical procedures — An inquiry into methods of evaluation A report of the International Commission on Radiological Protection and the International Commission on Radiological Units and Measurements Phys in Med Biol 2 (1957) 107 (Italian translation Societa Italiana di Radiologia Medica e di Medicina Nucleare Milan 1958)
Method of focal spot image formation and measurement (For diagnostic tubes up to 150 kvp) Acta radiol 55 (1961) 75 Amer J Roentgenol 85 (1961) 191 National Bureau of Standards Handbook No 78 p 80 U S Government Printing Office Washington 1961, Radiology 76 (1961), 121
Exposure of man to ionizing radiation arising from medical procedures with special reference to radiation induced diseases — An inquiry into methods of evaluation A report of the International Commission on Radiological Protection and the International Commission on Radiological Units and Measurements Phys in Med Biol 6 (1961) 199
Report of the RBE Committee to the International Commissions on Radiological Protection and Radiological Units and Measurements Hlth Phys 9 (1963) 357
BROWNELL G L BERMAN M and ROBERTSON J S Nomenclature for tracer kinetics Int J appl Radiat 19 (1968) 249
MACINTYRE W J FEDORUK S O HARRIS C C et coll Sensitivity and resolution in radioisotope scanning *In* Proceedings of the Symposium on Medical Radioisotopes Scintigraphy International Atomic Energy Agency Vienna 1969

ACTA RADIOLOGICA

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DENMARK FINLAND NORWAY AND SWEDEN

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HISTOPATHOLOGY OF LATE LOCAL RADIO-LESIONS IN THE GOAT BRAIN

by

BENGT ANDERSSON BORJE LARSSON LARS LEKSELL WILLIAM MAIR
BROR REXED PATRICK SOURANDER and JAN WENNERSTRAND

The use of stereotaxically directed high energy proton beams for the production of small circumscribed lesions in the depth of the brain has been demonstrated in previous investigations by LARSSON et coll 1958 LEKSELL et coll 1960 MAIR et coll 1967 In view of the current use of such techniques for local destruction of brain tissue in man (LARSSON et coll 1963) it is desirable to study the late appearance of local radio-lesions in the brain The present paper describes qualitatively 6 lesions in the brain of goats 1 1/2 to 4 years after cross-fire irradiation with a beam of 185 MeV protons The techniques of irradiation and dosimetry were except for some variation of the physical parameters the same as previously described in a short term study (LEKSELL et coll 1960)

Material and Methods

Radiation technique Adult goats of unknown age were irradiated by cross fire irradiation with 20 to 22 fields The individual field size number of fields

From the Department of Radiobiology the Gustaf Werner Institute and the Department of Anatomy University of Uppsala Sweden This investigation was supported by grants from the Swedish Medical Research Council Submitted for publication 22 October 1969

Table

Physical parameters of irradiation

Lesion No	Time of irrad (min)	Field size (mm²)	Number of fields	Number of planes	Max angle (0)	Real centre dose (rad)	Killed at (days after irrad)
1	115	7×2	21	2	20	20 000	573
2	59	7×2	21	2	20	20 000	757
3	61	7×2	21	2	20	20 000	781
4	119	7×2	21	2	20	20 000	845
5	45	10×3	21	2	20	15 000	1 170
6	47	7×2	21	2	20	20 000	1 453

angular coordinates, 'real centre dose' (cf LEKSELL et coll 1960), and time of irradiation are given in the Table above.

Animal care The animals were kept for study under ordinary farming conditions. During May to October they stayed out of doors in an enclosed well drained pasture with free access to water and salt. During November to April they were kept in a shed on straw. In the latter period they were given hay, salt and water freely, and in addition about 200 g of oats daily per animal. The animals were regularly observed but no neurologic examinations were made. Animals that died or were killed due to intercurrent disease were excluded from the investigation.

Histologic methods Brains taken from apparently healthy animals were studied at different intervals. The animals were decapitated 1 1/2 to 4 years after irradiation under nembutal anesthesia, and the brain was perfused through the carotids with physiologic saline followed by 5 per cent formol saline. The fixed brains were cut sagitally in slices 10 mm thick and examined macroscopically. The target region was embedded in celloidin and cut serially at 15μ. The following staining methods were used: Nissl, Woelke, Domagk, Romanes and Kossa.

Results

Lesion 1 18 months 20 000 rad The lesion was situated in the optic chiasma and adjacent brain tissue (Fig 1 upper). It took the form of a large necrotic cavity with a crenated margin lined by macrophages. Several smaller foci of similar appearance were present in the tissue around the main necrotic

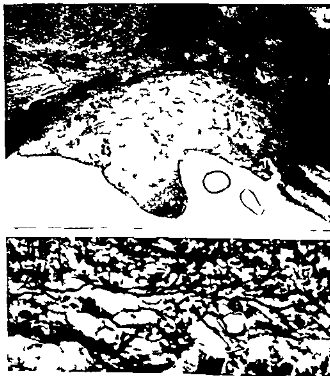


Fig 1 Lesion 1 18 months 20 000 rad *Upper* The lesion is sharply demarcated and l es in the optic chiasma and adjacent cerebral tissue Woelke ×8 *Lower* Swollen axons in the optic chiasma Romanes ×400

zone Increased vascularity with congested vessels perivascular round cell cuffing and proliferation of astrocytes were prominent around the cavity Some of the astrocytes were giant cells Some proliferation of astrocytes had also taken place in the damaged grey and white matter Some astrocytes were very large The optic chiasma contained swollen axons (Fig 1 lower) and was partially demyelinated and gliotic Some leptomeningeal vessels were collagenous and surrounded by lymphocytes

Lesion 2 25 months 20 000 rad The lesion which was situated in the thalamus showed a cavity with necrotic material and calcium concrements attached to its wall (Fig 2 upper) Capillaries in the tissue surrounding the cavity showed endothelial thickening and perivascular cuffing Giant cells (presumably abnormal glial cells) occurred at the edge of the lesion (Fig 2 lower)

Proliferation of astrocytes was seen in the wall of the cavity Some proliferat

Table

Physical parameters of irradiation

Lesion No	Time of irrad (min)	Field size (mm²)	Number of fields	Number of planes	Max angle (0)	Real centre dose (rad)	Killed at (day after irrad)
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Results

Lesion 1 18 months 20 000 rad The lesion was situated in the optic chiasma and adjacent brain tissue (Fig 1, upper) It took the form of a large necrotic cavity with a crenated margin lined by macrophages Several smaller foci of similar appearance were present in the tissue around the main necrotic

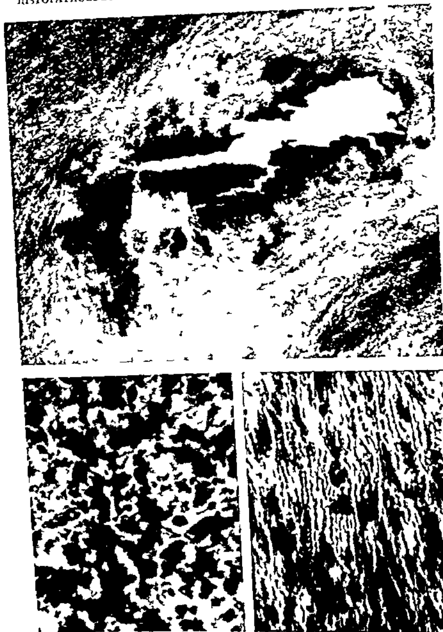


Fig 3 Lesion 3 26 months 20 000 rad *Upper* The lesion lies in the thalamus it consists of a glial scar with deposits of calcium Domagk ×20 *Lower left* Detail from calcium deposits in upper view Kossa ×200 *Lower right* Piloid astrocytes at the edge of the lesion Domagk ×200

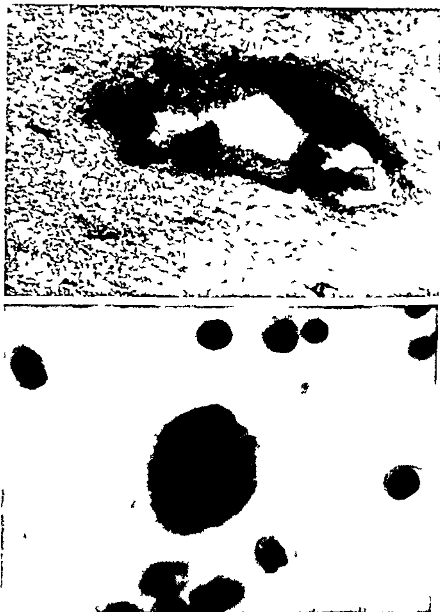


Fig 2 Lesion 2 25 months 20 000 rad *Upper* The lesion lies in the thalamus Deposits of calcium occur in the necrotic tissue and astrocyte proliferation at its edge where perivascular cuffing and round cells also occur Domagk × 70 *Lower* Giant cell with a very large nucleus at the edge of the lesion Domagk × 7[illegible]

ing astrocytes, occurring adjacent to a band of myelinated fibres just beyond the damaged zone, showed elongated nuclei lying parallel to one another Nerve cells also occurred close to the lesion Two very much smaller lesions lay adjacent to the main lesion both of them containing numerous astrocytes with



Fig 5 Lesion 5 39 months 15 000 rad The lesion lies in the optic tract and consists of a glial scar Adjacent tissue is normal Domagk ×8

several zones of proliferated astrocytes Some of the astrocytes took an arcuate form and the nuclei were elongated narrow and parallel to one another forming a palisade (piloid astrocytes) None of these cells were in mitosis (Fig 3, lower right) The blood vessels within the irradiated zone had thick collagenous walls and some had a thickened endothelial lining No evidence of new vessel formation hemorrhage or teleangiectasis was seen In the internal capsule and putamen there was a large necrotic zone and wide perivascular changes and astrocyte proliferation

Lesion 4 28 months 20 000 rad The lesion which was very large, was situated in the pallidum internal capsule and putamen Around the necrotic zone there was a reaction on the part of the astrocytes (Fig 4 upper) and the vessels showed perivascular cuffing with small round cells (Fig 4 lower) The lesion extended between fairly well preserved myelinated fibres and collections of nerve cells Calcium occurred at the edge of the necrosis in a layer of surrounding macrophages

Lesion 5 39 months 15 000 rad The irradiated zone lay in the optic tract and consisted of numerous astrocyte nuclei and glial fibres lying parallel to one another Around the gliosed tissue occurred well preserved large nerve cells Myelinated fibres lay adjacent to the lesion but there was no increase of astrocytes amongst these myelinated fibres No perivascular cuffing was seen in or around the lesion and there was no increase of vessels hemorrhage or teleangiectasis (Fig 5)



Fig 4 Lesion 4 28 months 20 000 rad *Upper* Astrocyte proliferation around the necrotic zone Domagk ×400 *Lower* Vessel showing perivascular cuffing with small round cells from the zone around the necrosis Domagk ×120

loosely arranged fibres and vessels showing perivascular cuffing Occasional giant cells also occurred

Lesion 3 26 months 20 000 rad The irradiated zone situated in the thalamus, consisted of a glial scar made up of astrocytes their fibres and some macrophages Deposits of calcium were present at the edge of the gliosis (Fig 3, upper and lower left) Nerve cells and some calcium deposits occurred among

1 Necrotic stage At a dose level of 20 krad this stage covers approximately the third and fourth weeks after irradiation when necrosis and acute degenerative and inflammatory reactions can be observed This stage has previously been studied in some detail (cf LEKSELL et coll 1960)

2 Stage of resorbtion This stage is characterized by resorbtion of cellular debris and beginning glial scar formation Phagocytic cells are here actively eliminating necrotic debris from the central part of the lesion Being maximal at the end of the necrotic stage (cf LEKSELL et coll 1960) this activity gradually decreases This stage is here represented by lesions Nos 1 2 3 and 4 It is generally characterized by astrocyte proliferation around the necrotic area and occasional giant cells which sometimes have large lobed nuclei Similar cells have been described by REXED et coll (1960) and by MONRO & MAIR (1958) This marginal zone also shows a chronic inflammatory reaction with congested vessels and formation of new capillaries often with endothelial thickening and round cell proliferation

In the marginal zone of lesions 2 and 3 an isomorphic gliosis with piloid astrocytes was seen In lesion 3, beginning glial scar formation could be observed This however was not the case in the two months older lesion 4 This might perhaps be explained by the fact that lesion 4 is larger and therefore the resorbtion phase had not progressed equally far

3 Late stage This stage seems to be characterized by prominent glial scar formation as demonstrated in Figs 5 and 6 from lesions 5 and 6 respectively These lesions show no inflammatory reaction no giant cells no increase of vessels no teleangiectasis and no hemorrhage There has been no evidence in this study of cellular elements resembling those of neoplasms in an early stage Neither have any teleangiectasis or bleedings been observed It may be concluded from the findings presented here that there are no untoward late complications in the goat brain after irradiation of the type described It should be understood that the material on which this conclusion is based is small and also that the conclusion may be valid only in the species under study

Since no cellular elements resembling those of neoplasms in an early stage were seen it gives some support to the belief that the risk of tumour formation may be minor

SUMMARY

Lesions in the depth of the goat brain were produced by cross-fire irradiation with high energy proton beams and a long term study was performed to determine the late histopathologic effects No late untoward changes such as cellular elements resembling those of neoplasms in early stage teleangiectasis and hemorrhage were observed

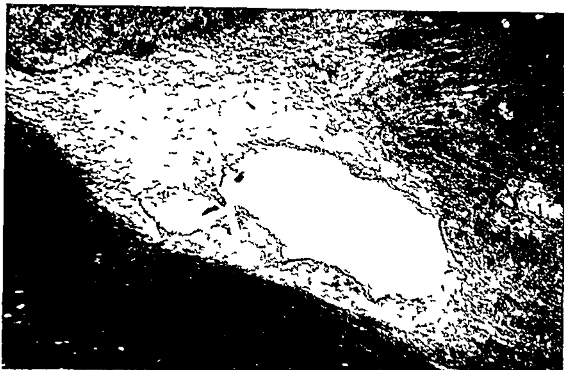


Fig. 6. Lesion 6. 48 months. 20 000 rad. The lesion lies in the internal capsule and consists of a cavity surrounded by a glial scar. Woelke. ×10.

Lesion 6. 48 months. 20 000 rad. A well defined ellipsoid lesion was situated in the internal capsule. In the centre there was a glial scar containing a cavity (Fig. 6), and around this scar myelinated fibres and nerve cells with an astrocyte reaction. There was no perivascular cuffing and the astrocytes were not of the large size seen in the earlier lesions.

Discussion

The aim of this investigation was to see whether any late untoward reactions occur in animals irradiated by the same technique as used in radiosurgery of the brain.

Pilot studies, in man, with lesions made in the thalamus for parkinsonism and in the mesencephalon for pain and for pain or psychiatric disturbances in the frontal lobes are made (Leksell 1961, Larsson et coll. 1963, Kjellberg et coll. 1964, and Leksell 1966). The observations also give a qualitative picture of the characteristics of radio lesions in the brain after irradiation. The phase which follows upon the acute degenerative phase may be divided into three stages.

FROM RADIUMHEMMET AND THE UROLOGY CLINIC KAROLINSKA SJUKHUSET, STOCKHOLM SWEDEN

INSTILLATION OF THIO TEPA (TIFOSYL) IN VESICAL PAPILLOMATOSIS

by

F EDSMYR and J BOMAN

The treatment of vesical papillomatosis is a serious problem in urology Transurethral excision or coagulation are the conventional methods but the frequency of recurrence is high and repeated treatments are often necessary Papillomata may sometimes be so widespread inside the bladder that electro coagulation at one or several lesions may be inadequate as a method of treatment Attempts have been made to instil radioactive solutions through a rubber bag or directly into the bladder to destroy the papillomata and ^{60}Co ^{8}Br ^{198}Au and ^{76}As have been employed to this end (EINHORN et coll 1964) The results have not been satisfactory Recurrence and complications such as contracted bladder haematuria and vesico ureteral reflux are not uncommon High radiation doses are necessary to destroy the papillomata but these may in turn result in damage to surrounding healthy tissue

The use of chemotherapeutic agents topically in the bladder has long been employed in cases of widespread vesical papillomatosis The various chemotherapeutic agents injected have included silver nitrate (HERRING 1903) podophyllin (SEMPLE 1948 KELLY & HARTWELL 1954) and over the last ten years tifosyl (Thio tepa) (JONES & SWINNEY 1961 ESQUIVEL et coll 1965 VEENEMA

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ZUSAMMENFASSUNG

Läsionen in der Tiefe von Ziegengehirnen wurden durch Kreuz-Feuer-Bestrahlung mit Hochenergie-Protonstrahlen hervorgerufen und eine Langzeitstudie, um die späten histopathologischen Veränderungen festzustellen, wurde durchgeführt. Keine ungünstigen späten Veränderungen, wie Zellenelemente ähnlich denen bei Neoplasmen in frühen Stadien, Teleangiektasien oder Blutung, wurden beobachtet.

RÉSUMÉ

Les auteurs ont utilisé une irradiation à feux croisés par des faisceaux de protons de haute énergie pour produire des lésions dans la profondeur du cerveau de la chèvre et ont fait des études à long terme pour déterminer les effets histopathologiques éloigné de cette irradiation. Ils n'ont pas trouvé de modifications défavorables tels que des éléments cellulaires ressemblant à ceux des néoplasies au stade précoce, des télangiectasies et des hémorragies.

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FROM RADIUMHEMMET AND THE UROLOGY CLINIC KAROLINSKA SJUKHUSET STOCKHOLM, SWEDEN

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F. EDSMYR and J. BOMAN

The treatment of vesical papillomatosis is a serious problem in urology. Transurethral excision or coagulation are the conventional methods but the frequency of recurrence is high and repeated treatments are often necessary. Papillomata may sometimes be so widespread inside the bladder that electro-coagulation at one or several sessions may be inadequate as a method of treatment. Attempts have been made to instil radioactive solutions through a rubber bag or directly into the bladder to destroy the papillomata, and ^{60}Co, ^{82}Br, ^{198}Au and ^{76}As have been employed to this end (EINHORN *et coll* 1964). The results have not been satisfactory. Recurrence and complications such as contracted bladder, haematuria and vesico-ureteral *reflux* are not uncommon. High radiation doses are necessary to destroy the papillomata but these may in turn result in damage to surrounding healthy tissue.

The use of chemotherapeutic agents topically in the bladder has long been employed in cases of widespread vesical papillomatosis. The various chemotherapeutic agents injected have included silver nitrate (HERRING 1903), podophyllin (SEMPLE 1948, KELLY & HARTWELL 1954) and over the last ten years tifosyl (Thio-tepa) (JONES & SWINNEY 1961, ESQUIVEL et coll 1965, VEENEMA

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Table

Summary of the results of tifosyl treatments reported in the literature

Authors and year of publication	Number of patients	Results	Papilloma	Carcinoma stages			
				A	B	C	D
JONES & SWINNEY 1961	16	No effect	2	—	—	2	—
		Partial destruction	7	—	—	1	—
		Complete destruction	4	—	—	—	—
VEENEMA et coll. 1962	57	No effect	11	—	3*	—	[illegible]
		Partial destruction	14	—	7*	—	2
		Complete destruction	13	—	—	—	—
ESQUIVEL, MACKENZIE & WHITEMORE 1964	20	No effect	3	—	6	1	2
		Partial destruction	2	2	2	1	—
		Complete destruction	3	—	—	—	—

* includes those who received prior radiotherapy

et coll. 1962). The results of tifosyl treatment reported in the literature are summarized in the accompanying Table.

We have used tifosyl instillation since 1963 but since 1967 in accordance with special principles and unitary doses. Twenty nine patients with widespread papillomatosis have had installations of tifosyl (Thio tepa) into the bladder. Eight of these were women and twenty one were men between 46 and 80 years of age. The average period of time for the papillomatosis before tifosyl treatment was 5.3 (1 to 17) years and repeated coagulations had been performed for recurrence.

Method. A complete series of treatments consisted of six installations of tifosyl in doses of 50 mg per instillation. Treatment was given on alternate days, usually in the *out patient department*. The patients assumed several positions during the treatment in order to ensure complete contact between the bladder wall and the active agent. The patients were asked to keep the solution (50 ml) in the bladder for two hours. To avoid dilution of the solution in the bladder, the fluid intake was restricted 12 hours before treatment.

Results

A clinical grading of the effect of treatment on the papillomata was carried out during the cystoscopies which were performed 4 to 6 weeks after the tifosyl treatment A further series of treatments was usually given if no effect was observed after the first one No further treatment was given if regression of the papillomata had occurred No coagulation of the remaining papillomata was performed until it was felt that no further regression could be expected

The clinical cystoscopic results were codified 0 to 3 where zero indicates no effect or progress 1 indicates reduction of necrosis of the papillomata 2 stands fore one or more papillomata gone and 3 for all papillomata gone The following results were obtained

Codes	0	1	2	3	*Total*
One series	4	—	4	6	14
Two series	1	1	3	4	9
Three series	—	—	4	2	6

Complete regression occurred in twelve out of twenty nine cases (41 %) even of these occurring directly and five others after coagulation of some small residual papillomata Complete regression or almost complete regression (codes 2 and 3) occurred in twenty three out of the twenty nine cases (80 %) No effect could be observed in five (17 %) of the cases

The number of treatment series in the different groups are given below

	Number of patients	Series given
Code 0	4	1
	2	2
Code 1	1	2
Code 2	4	1
	3	2
	4	3
Code 3	6	1
	4	2
	2	3

The observation period starting with the first treatment with tifosyl averaged one year (between 2 months and 5 years) The period is too short to allow evaluation of the long term frequency of recurrence It seems however as if a complete series of tifosyl treatments makes it easier to control the remaining papillomata by coagulation

Results related to the primary papilloma size In principle, only those patients were chosen for tifosyl therapy who at cystoscopy had thin, slender papillomata spread over the mucous membrane of the bladder at the sites of multiple previous electrocoagulations There had been much difficulty in effectively destroying new recurrent papillomata by electrocoagulation, and instillation therapy with tifosyl had therefore been adopted The results are tabulated below according to the size of the lesion

Codes	0	1	2	3	Total
'Carpet (widespread papillomatosis)	4	—	8	9	21
< Pea size	—	1	2	3	6
= Pea size	—	—	1	—	1
> Pea size	1	—	—	—	1

Results related to the histologic grade of malignancy Biopsies were performed in all cases (except one) before treatment, where the histologic malignancy was grade I or II, as shown below

Codes	*0*	*1*	*2*	*3*	*Total*
Grade MI	2	1	7	6	16
Grade MII	3	—	4	5	12
Grade 0	—	—	—	1	1

There was no difference in the results of treatment between the two grades of malignancy

Results related to bladder infection An adequate bacteriologic investigation was not made in connection with the treatment in twenty one cases in which the results could only be judged by the clinical condition of the patient Only one of the other eight cases developed infection of the bladder after instillation of tifosyl and this patient experienced no discomfort The others either had no trouble with constriction or infection, or it was slight Prophylactic antibiotic therapy was used in connection with the treatment, as related below

Codes	0	1	2	3	Total
Infection before and after treatment	1	—	2	1	4
Infection before treatment only	—	1	—	—	1
Infection after treatment only	—	—	—	1	1
No infection	1	—	—	1	2
Not investigated	3	1	8	9	21

Complications Four of the twenty nine patients treated with tifosyl had complications that might be considered as side effects In one man the tifosyl treatment was interrupted after five instillations because of pain and discomfort in the bladder One woman became tired and had small haemorrhages in the skin of the lower parts of the legs and experienced giddiness three weeks after a tifosyl instillation series the lowest thrombocyte value was 85 000 all the symptoms disappeared later The third patient was a man who had a decrease in the thrombocyte values from 186 000 to 47 000 without symptoms or signs in connection with the treatment series the values returned to normal later The fourth patient was a woman aged 71, who had had suprapubic cystotomy seven years earlier with resection of a walnut sized papilloma Five years later the bladder was covered with papillomata Eight coagulations were performed one of them in connection with the sectio alta One year before the instillation of tifosyl perforation of the bladder occurred in connection with coagulation and biopsy of the papillomata Dilatation of the left ureter was present and nephroureterectomy revealed a papilloma in the ureter postoperative ileus required laparotomy Multiple papillomata of the bladder occurred 6 months later Coagulation of as many papillomata as possible was carried out and tifosyl was instilled ten times in doses of 50 mg per instillation every second day The day after the last instillation the white blood cell count was 9 000 and the thrombocyte count normal Four days later the white blood cells decreased from 2 500 to 50 for a few days and the thrombocytes from 10 000 to 3 000 The patient died of sepsis probably caused by pancytopenia Autopsy revealed no tumour or papillomatous tissue in the bladder but marked cystitis with ulceration The period between the coagulation and the instillation of tifosyl was probably too short and the drug was carried into the blood stream via the ulcerated vesical mucous membrane

SUMMARY

Twenty nine cases of widespread vesical papillomatosis were treated by the instillation of tifosyl The method is described Complete or almost complete regression was achieved in twenty three of the cases

ZUSAMMENFASSUNG

Spülungen mit Tifosyl zur Behandlung ausgebreiteter Papillomatose der Harnblase wurden an neunundzwanzig Fällen verwandt Die Methode wird beschrieben In dreiundzwanzig Fällen erfolgte komplette Heilung oder wenigstens ein beinahe vollständiger Rückgang der Erkrankung

RÉSUMÉ

Trente neuf cas de papillomatose vesicale etendue ont ete traites par instillation de tifosyl Description de la méthode On a observé une regression complete ou presque complete dans vingt trois de ces cas

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FROM THE RADIUM CENTRE (DIRECTOR SIGVARD KAAE) AND THE E N T DEPARTMENT (DIRECTOR H C ANDERSEN), MUNICIPAL HOSPITAL OF THE UNIVERSITY OF ARHUS DENMARK

CARCINOMA OF THE LARYNX

I Treatment mainly by primary irradiation

by

KARSTEN JORGENSEN

Carcinoma of the larynx was treated almost exclusively by surgery in the early part of this century and then only to a limited extent In the 1920 s COUTARD (1932) developed fractionated roentgen irradiation for carcinoma of the pharynx and larynx This method adopted in Denmark and modified by JENS NIELSEN (1942) has opened up a possibility of curative treatment of laryngeal carcinoma by primary radiotherapy The procedure has gradually gained ground in a number of European centres while in U S A the condition is still treated mainly by primary surgery

The present report deals with the results obtained chiefly by primary radio therapy sometimes supplemented by surgery These results will be compared with those from centres where surgery is mostly employed

Material A total of 263 patients was examined and treated for carcinoma of the larynx during the period 1942—1963 The review runs to the middle of 1963 when ^{60}Co kilocurie therapy began to be used It covers only 248 of

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Table 1

Number of cases (total 248) treated for carcinoma of the larynx during the separate 5-year periods from 1 January 1944 up to 1 August 1963 and the crude 5-year survival rates

	Number of cases	Crude 5 year survival rates
1 January 1944—1 January 1949	27	44 %
1 January 1949—1 January 1954	46	65
1 January 1954—1 January 1959	69	67 %
1 January 1959—1 August 1963	106	65 %
1 January 1944—1 August 1963	248	63 %

the patients, fifteen having been excluded because of recurrence following treatment elsewhere (five), histologic examination not performed (five), doubt about the diagnosis on review of the histologic specimens (three), transferred for treatment elsewhere (two)

A few of the 248 patients received minor, palliative doses of radiation and in others radiotherapy was abandoned after some days because of senile debility, general weakness and similar reasons Apart from the exclusion of the fifteen patients listed above, the series may be considered unselected The follow up was 100 %

As to the incidence, CLEMMESEN (1965) has reported that carcinoma of the larynx made up 0 91 % of all the cases of carcinoma notified in Denmark from 1943 to 1957 From 1943 to 1948 about 35 new cases were notified annually and from 1953 to 1957 about 60 cases This increase is considerably in excess of what might have been expected from the increase in the total number of carcinoma cases, so that the incidence of laryngeal carcinoma must be augmenting Indeed, this is reflected in the present series in which there was a appreciable increase in the number of new cases during the separate 5 year periods (Table 1)

As regards the sex ratio and the age distribution in the present series, 11 % (27/248) of the patients were females and 89 % (221/248) were males The average age at the time of the histologic diagnosis was 61 years 5 months, as indicated in Fig 1 The youngest patient was a woman of 27, the oldest a man of 87

The histologic type was squamous cell carcinoma in 247 cases while one was described as a case of adenocarcinoma, possibly adenomyoepithelioma Seven of the 247 squamous cell carcinomas were of the stage carcinoma in situ

The symptoms and signs consisted of hoarseness as initial symptom in 86 % in 9 % there was irritation of the throat, at times with pain radiating to the ear In the remaining 5 % of the cases, the initial symptoms and signs were equally

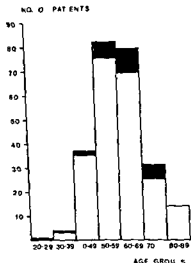


Fig 1 Age distribution at the time of the histologic diagnosis black fields indicating the number and ages of the women and the white columns those of the men in the series

distributed between dysphagia enlargement of the cervical lymph nodes cough and dyspnoea The interval from the onset of the initial symptom until the histologic diagnosis was less than 4 months in 41 % 4 to 8 months in 28 % and more than 8 months in 31 % of cases

Classification This was made according to the TNM system in the UICC modification The rules of classification are apparent from the following survey (UICC 1968) of the group T Primary tumour

Supraglottic	*Glottic*	*Subglottic*
T1—Tumour confined to one anatomical site within the larynx		
Tumour confined to laryngeal surface of epiglottis or to an aryepiglottic fold or to a ventricular cavity or a ventricular band	Tumour confined to one vocal cord and mobility of cord remains normal	Tumour limited to one side of the subglottic region exclusive of the undersurface of cord
T2—Tumour confined to one anatomical region within the larynx		
Tumour involving the epiglottis extending to the ventricular cavities or bands	Tumour involving both cords with normal mobility of one or both cords with fixation of cord(s)	Tumour extending to two sides of subglottic region exclusive to the undersurface of cords

T3 — Tumour extending beyond one anatomical region within the larynx

Tumour of the epiglottis and/or ventricles or ventricular bands and extending into the cords	Tumour extending from cords either to subglottic region or to supraglottic region i.e. to ventricular bands or ventricles	Tumour involving subglottic region and extending on to the cords

T4 — Tumour extending beyond the larynx

Tumour as in T1 T2 or T3 but with direct extension to piriform sinus post cricoid region vallecula or base of tongue	Tumour as in T1 T2 or T3 but with direct extension through cartilage to the piriform sinus or to the postcricoid region	Tumour as in T1 T2 or T3 but with direct extension to trachea skin or post cricoid region

N — *Lymph nodes*

N0 — No lymph nodes palpable
N1 — Movable homolateral nodes
N2 — Movable contralateral or bilateral nodes
N3 — Fixed nodes

M — *Distant metastases*

M0 — No evidence of distant metastases
M1 — Distant metastases present

The distribution of the lesions by site and extent is apparent from Table 2 To restrict the number of groups, cases having presumed lymph node metastases were divided into three groups a supraglottic, a glottic, and a subglottic group, regardless of whether these were T1, T2, or T4 cases It will be seen that only 22 cases (9 %) had presumed lymph node metastases in the neck on admission The incidence of lymph node metastases in the three main groups was as follows supraglottic seventeen cases (27 %), glottic four (2 %), and subglottic one case (8 %)

Method of treatment A total of 217 cases were treated primarily by irradiation and 29 primarily by surgery Conventional roentgen radiation was used throughout the period 1944—1963 From the beginning of 1959 up till August 1963 most cases were treated by a ^{60}Co unit whose radiation source was of decicurie strength, so that during the last 5 year period the irradiation was administered by two different methods The small field size of the ^{60}Co unit made it suitable for treating fairly small neoplasms while the conventional roentgen apparatus was employed mainly for larger growths and lymph node metastases

Table 2

Site of 248 cases of carcinoma of the larynx grouped according to the TNM system (UICC)

	Supra glottic	Glottic	Sub-glottic	Total
T1N0M0	8	81	—	89
T2N0M0	9	32	—	41
T3N0M0	21	53	9	83
T4N0M0	7	3	3	13
T1+2+3+4 N1+2+3M0	17	4	1	22
Total	62	173	13	248

Conventional roentgen radiation was administered to two opposed fields, one field being treated each day An extra field was added anteriorly however if necessitated by a large growth The field size was usually 6 cm × 6 cm but was extended when indicated by the size of the neoplasm or by lymph node metastases The tumour dose was generally about 6 000 R over a total treatment period of 30 to 35 days up till 1950 the dose was somewhat larger and the period longer

A ^{60}Co unit of 10 to 15 decacurie was generally used during the period 1959—1963 (maximum field size 3 cm × 3 cm FSD 10 cm) Small growths of the vocal cords were irradiated to two anterolateral fields using a wedge filter with its base medially The calculated tumour doses were usually 5 000 to 7 000 R and the treatment period 25 to 35 days

Two of the twenty nine cases treated primarily by surgery had endoscopic removal of very small carcinomas of the vocal cords Apart from these two cases the indications for primary surgery were large extension of the tumour a large mass causing respiratory obstruction or a growth invading or penetrating the laryngeal cartilage Involvement of the hypopharynx or subglottic location in cases of not too advanced age and in good general condition were also indications for primary surgery (only total laryngectomy) All the operations were performed by the same surgeon (H C Andersen)

Follow up All the patients were examined by the radiotherapists and otolaryngologists at weekly conferences. After completed irradiation the subjects attended as out patients first at intervals of 2 to 3 months Any suggestion of residues at the termination of radiotherapy resulted in the patients being admitted about 6 to 8 weeks later for examination by direct laryngoscopy and

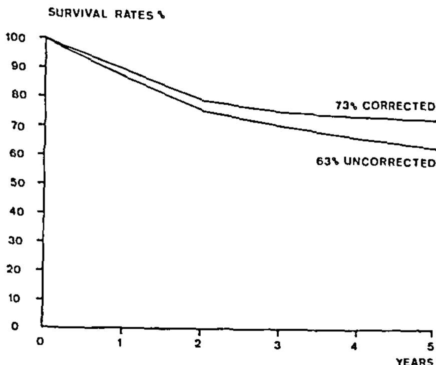


Fig. 2. Decrement curves for 248 patients with carcinoma of the larynx. The upper curve is corrected for mortality from other causes, the lower curve is uncorrected.

biopsy. A positive biopsy usually meant that the patient was subjected to operation (a few refused and some were too weak). Patients without evidence of residues or recurrence were followed for 10 years, but after the first 3 years were seen only once a year.

Results

The 5-year survival rates for all the patients are listed in Table 1 and divided into 5-year periods. The results have been almost stationary for 15 years.

Fig. 2 illustrates the total result depicted in two decrement curves. The lower, uncorrected curve indicates the calculation of the total mortality coefficients for each individual year. The upper curve is produced by the calculation of the corrected mortality coefficients for the separate years, eliminating other causes of death. The latter coefficients were calculated from the formula

$$q_n = \frac{c}{r/2 + c + l}$$

(NOHRMAN 1953), where q_n is the corrected mortality coefficient in the nth year, c the deaths from the condition occurring in the nth year, r the number

Table 3

Subdivision of the 248 cases of carcinoma of the larynx according to the type of primary treatment given

Primary treatment	Number of cases	5 year survivals recurrence free	Deaths	Alive with recurrence	Crude 5 year survival rates
Irradiation	219	140	78	1	64
Surgery	29	15	13	1	55
Total	248	155	91	2	63

Table 4

Results of treatment in 248 cases of carcinoma of the larynx subdivided into the three main locations

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
Supraglottic	62	18 3	13/25	2/5	33/62	53
Glottic	173	82/117	26/40	9/16	117/173	68
Subglottic	13	1/3	1/2	5 8	7/13	54
Total	248	101/152	40/67	16/29	157/248	63

IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

of deaths due to other causes and *l* the number of patients alive at the end of the year concerned The corrected curve thus represents the cancer deaths most of which occurred during the first two years The curve is almost horizontal after the fourth year there being only a few late deaths from carcinoma

The types of primary treatment are grouped in Table 3 A comparison of the surgical and radiologic treatments is out of question as the former consisted of only a few relatively advanced cases and was thus a special selected group

Table 4 gives the results in the three main groups Neoplasms arising from the vocal cords proper apparently carry the most favourable prognosis

Supraglottic group Table 5 indicates that the prognosis for T1 + 2 + 3N0 is good The explanation of the supraglottic group as a *whole* being in the most unfavourable situation is the relatively high incidence of lymph node metastases and the relatively large number of T4N0M0 cases

Table 5

Results of treatment in 62 cases of supraglottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
T1N0M0	8	6/7	1/1	—	7/8	71 %
T2N0M0	9	2/5	3/4	—	5/9	
T3N0M0	21	6/9	8/10	1/2	15/21	
T4N0M0	7	0/3	0/2	1/2	1/7	25 %
T1+2+3+4 N1+2+3M0	17	4/8	1/8	0/1	5/17	

* IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

Table 6

Results of treatment in 173 cases of glottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
T1N0M0	81	50/63	14/16	2/2	66/81	81 %
T2N0M0	32	15/24	4/7	1/1	20/32	63 %
T3N0M0	53	17/26	8/17	5/10	30/53	57 %
T4N0M0	3	0/1	—	1/2	1/3	1/3
T3+4 N1+2+3 M0	4	0/3	—	0/1	0/4	0/4

* IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

Glottic group The results are presented in Table 6 and illustrated by decrement curves in Fig 3 by the same method of calculation as mentioned above The two top curves in Fig 3 indicate that the T1 group had a relatively high *mortality* from causes other than malignancy Seventeen of the thirty two T2 cases had fixed vocal cords and fifteen mobile cords The latter

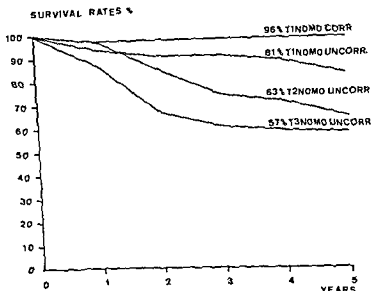


Fig 3 Decrement curves for patients with carcinoma of the glottis The uppermost curve is corre ted for mortality from causes other than carcinoma of the larynx and all the other curves are uncorrected

category were cases in which the tumour involved both cords but mobility was preserved The crude 5 year survival rates for these two groups were cases with fixed cords 59 % (10/17) cases with mobile cords 71 % (10/14) Further mention will be made of this difference later and the results in the T3 group will al o be considered in greater detail in the discussion

The prognosis with fixed cords in the 173 cases of carcinoma of the glottis was assessed A total of 58 cases had fixed cords and 115 had freely mobile vocal cords The 5 year results for these two groups were 52 % (30/58) and 76 % (87/115) respectively

Subglottic group The results for this group are listed in Table 7 The number of cases was small but the indication seems to be that radiotherapy alone has little chance of effecting a cure

Comparison of the results of roentgen and ^{60}Co (decacurie unit) radiation It is of considerable interest to compare the 160 to 250 kV apparatus with the ^{60}Co unit of decacurie strength as regards 5 year survivals without other treatment For this purpose it was decided to compare cases treated primarily by ^{60}Co during the period 1959—1963 with those which received primary

Table 7

Results of treatment in 13 cases of subglottic carcinoma of the larynx subdivided according to the TNM system (UICC)

	Number of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	IRR + SURG* for recurrences and res	Primary surgery	Total	
T3N0M0	9	0/2	1/2	4/5	5/9	5/9
T4N0M0	3	1/1	—	1/2	2/3	2/3
T3N1M0	1	—	—	0/1	0/1	0/1

* IRR = irradiation SURG = surgery after failure of irradiation res = residual tumour

roentgen irradiation during the period 1949—1963 A considerably larger number of T4 and N1 + 2 + 3 growths were present in the latter group, and these were therefore excluded from both groups This leaves only T1, T2 and T3N0M0 cases Thus curtailed, the groups are fully comparable as regards classification, sex ratio, and age distribution The difference in time will be disregarded as it is probably of no great importance

A total of 70 cases treated with the ^{60}Co decacurie unit are compared in Table 8 with 97 cases treated by the conventional roentgen apparatus A striking difference was apparent between these two groups in the cure rates after irradiation as the only treatment Indeed, statistical calculation disclosed that the difference was distinctly significant It is apparent from the table that there was but little difference between the crude 5 year survival rates in the two groups (71 4 and 74 2 %, respectively), this being due to two factors (1) a relatively far larger number of the ^{60}Co group had a secondary operation, (2) the surgical results in the ^{60}Co group were considerably better than in the roentgen group This is because ^{60}Co therapy also failed in a number of cases with small vocal cord carcinoma which have a favourable prognosis after secondary surgery (Nearly all the partial laryngectomies were in the ^{60}Co group, cf Table 9)

The inefficacy of the ^{60}Co unit (decacurie) might seem surprising There are at least two explanations The relatively weak radiation source with a focus skin distance of 10 cm and a field size of 3 cm × 3 cm did not afford an entirely expedient course of the isodose curves This combined with an inaccurate adjustment technique, *is presumably the reason why the calculated* tumour doses were not attained in a number of instances

Table 8

Results in two comparable groups of cases one treated with the ⁶⁰Co unit and the other with the conventional roentgen apparatus

	5 year cure rates after irradiation as only treatment		Percentage and number of cases subjected to secondary surgery		Results of secondary surgery at the 5 year limit		Crude 5 year survival rates	
Co-group 70 cases	38 6	(27/70)	41 0	(29/70)	79 3	(23/29)	71 4	(50/70)
Roentgen group 97 patients	58 7	(57/97)	24 7	(24/97)	62 5	(15/24)	74 2	(72/97)
Level of significance	Standard error of the difference 7 48 difference 2 7 times the standard error		Standard error of the difference 5 84 difference 2 5 times the standard error				Standard error of the difference 6 96 difference 0 4 times the standard error	

Results of surgery A total survey of the surgical procedures as well as the 5 year survivals from the institution of the primary treatment is given in Table 9 Out of 22 partial laryngectomies eight were done because of malignant residues and fourteen for recurrence At a 5 year limit the survival was 4/8 and 13/14 respectively indicating that partial laryngectomy is less suited for the treatment of residues During the period under discussion a total of 75 total laryngectomies were carried out 27 primary and 48 secondary for residues or recurrence Of these 75 patients forty were alive at the 5 year limit It must be mentioned that laryngectomy and dissection of cervical lymph nodes were not always performed at the same session As for the twenty five cervical node dissections the histologic examination revealed metastases in nine and non malignant changes in thirteen patients The result was not stated in three patients All nine patients with positive histologic findings died while all but one of the histologically negative patients survived The indication for cervical node dissection was palpable and doubtful lymph nodes on admission or in the course of the laryngectomy

Vocal quality after radiotherapy and surgery The quality of the voice was assessed from data in the case records of patients who survived for more than 5 years after irradiation as only treatment The voice was normal or almost normal in 90 % (66/73) of the roentgen irradiated patients The corresponding values for the ⁶⁰Co group were 82 % (23/28) The fourteen patients subjected to partial laryngectomy all of whom had passed the 5 year limit were articulate

Table 9

Operations (a total 103) performed in 97 of 248 patients treated during the years January 1944 — inclusive of July 1963 — 29 primary surgery — 73 for residues or recurrences — 1 perichondritis

	Supra glottic	Glottic	Sub glottic	Total	At 5 year limit	
					Alive	Dead
Endoscopic excision	—	2	—	2	2	—
Laryngofissure with cordectomy	—	1	—	1	1	—
Partial laryngectomy	3	13	—	16	14	2
Partial laryngectomy with laryngectomy later	2	3	1	6	3	3
Total laryngectomy	13*	26	8	47	23	24
Total laryngectomy and neck dissection	10	11	1	22	14	8
Neck dissection alone	3	—	—	3	—	3
Total	31*	56	10	97	57	40

* including the patient operated for perichondritis

although their voices were rough and hoarse. Vocal function in the patients who underwent total laryngectomy has been studied by JENSEN & BALSLEV (1967) who reported that about 60 % attained satisfactory oesophageal voices. A few used a voice vibrator.

Side effects of radiotherapy. Acute radiodermatitis of varying degree was observed in all the roentgen irradiated patients, whereas those irradiated with ^{60}Co exhibited only mild cutaneous reactions. Chronic cutaneous changes of a more serious nature did not occur.

All the irradiated patients developed more or less marked epithelitis of the larynx. More severe reactions resulting in oedema were but few, and only two patients required tracheotomy during the treatment, both these had large T3 glottic tumours with a cramped space. Chronic mucosal complaints with dryness and tenderness of the pharynx occurred in seven patients three of whom, however, reported that these had disappeared after about 5 years.

Perichondritis, presumably induced by irradiation, was observed in five patients, three of those treated by ^{60}Co and two by roentgen irradiation. One patient had to have laryngectomy, two tracheotomy, and two patients had severe symptoms for about 2 years. Apart from these, a few patients had perichondritis due most probably to a recurrence of the neoplasm.

Complications and sequelae of surgery. The complications in 103 operations were few. There were no deaths or serious sequelae during the operation or in

Table 10

Relations between duration of initial symptom stage of disease and rate of survival

Duration of symptoms before histological diagnosis	0—3 months	4—7 months	8—11 months	12—15 months	16—19 months	More than 20 months	No information
Number of patients	101	69	15	18	17	23	5
Crude 5 year survival rates	65/101 64	43/69 62	9/15 60	10/18 56	9/17 53	18/23 78	3/5
Groups T1+2/T1+2+3+4	(64/101) 63	(40 69) 58		(21/50)44		9/23	3/5

the first few postoperative weeks Apart from small suture leaks fistulas to the hypopharynx of short duration occurred in seven of the totally laryngectomized patients Chronic fistulas requiring one or more operations, occurred in four patients and several patients had plastic stoma repair

One of the partially laryngectomized patients had to be fitted with a cannula because of stenosis and one complained of slight stridor on exertion The special problems of the totally laryngectomized patients such as dyspnoea discharges and social problems have been thoroughly discussed by JENSEN & BALSLEV

Prognosis in relation to duration of the initial symptom As already mentioned there were quite marked differences in the intervals from the onset of the first symptom until the histologic diagnoses It is of interest to assess the prognostic role of this factor The entire series was therefore divided into groups according to whether the histologic diagnosis was made 0—4 5—7 8—12 or more months after the onset of the initial symptom (Table 10) The longer the interval the poorer the prognosis

Table 10 also indicates the staging in relation to the duration of the initial symptom The total numbers of T1 and T2 cases are given as percentages of the total numbers for the separate interval groups As might be expected this reveals that a long duration of symptoms produced relatively fewer T1 and T2 cases or in other words a late diagnosis means a considerably larger number of advanced cases (T3 and T4 cases)

The group of cases in which the initial symptom had been present for more than 20 months differs entirely from what might have been expected The explanation may be that the majority in this group consisted of cases with pre existing chronic laryngitis although no exact data or values can be given This group contains a relatively large number of cases classified as advanced Considering the extremely favourable prognosis it seems justified to conclude that carcinoma

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indicate that these occurred mainly within the first two years The practical consequences must be that materials analysed in this way afford an accurate expression of the efficacy of treatment in a series provided a reasonable number of cases have been followed for more than 3 years The special factors applying to the three main groups will now be discussed

The treatment in the supraglottic group is complicated mainly by the high incidence of lymph node metastases Dissection of the cervical lymph nodes with histologic evidence of lymph node metastases in nine cases did not lead to any cure whereas four cases with presumed metastases were cured by irradiation The tendency in recent years in centres using surgery as primary treatment seems to have been cervical node dissection in dealing with supraglottic carcinoma even when no lymph nodes are palpable but paying regard to the extent of the tumour (JACKSON & NORRIS 1962 MCCOMB 1966)

The results within the group of glottic T1 cases are in the same range as reported from elsewhere (MCCABE et coll 1960 CHABAZIAN et coll 1967 PEREZ 1968) An attitude to treat this group primarily by irradiation appears to exist in most centres

The glottic T2 cases may be classified as already mentioned, into two subgroups with or without fixation of the vocal cords and these subgroups presented a distinct prognostic difference (59 % and 71 % crude 5 year survival rates respectively) HEISE & BAYLIS, dealing with the same problem reported a similar prognostic difference between the two subgroups 57 % for fixed and 70 % for unfixed cases On this basis it would seem reasonable to suggest that the T2 group be divided into two subgroups T2 + fix with fixation of cord(s) and T2 — fix without fixation of cord(s) It is tempting to transfer T2 + fix to the T3 group but probably not permissible since all T2 + fix cases cannot be said with certainty to be more extensive or more deeply infiltrating than T2 — fix cases

Another factor is the prognostic role of fixed vocal cords in all glottic carcinomas considered together The survival rates for the two groups with fixed and unfixed vocal cords in this series were 52 % and 76 % respectively This finding is not surprising considering that fixation is presumably usually a sign of more deeply infiltrating processes The prognostic role of vocal cord fixation has been discussed by SVANE KNUDSEN (1960) RYGARD & HANSEN (1966) and MARTENSSON et coll (1967)

Glottic T3 cases from our series appear to be comparable with similar cases treated in other centres where surgery is the primary therapeutic principle The results are largely the same with crude 5 year survival rates of a little below 60 % The frequency of total laryngectomy in the surgical materials is about 85 % (SMITH et coll 1961) while in our series it was 43 % (23/53) Of the

in cases with chronic laryngitis is to some extent erroneously placed in an advanced group

Discussion

Investigation of the symptoms revealed that the initial symptom of laryngeal carcinoma was hoarseness in 86 % of cases By way of comparison, it may be mentioned that in a series of 1 498 cases BALZELI & PUTNEY (1954) gave this percentage as 85 5 In more than half the number of our cases the diagnosis was reached more than 4 months after the onset of the initial symptom Table 10 indicates that a late diagnosis makes for a poorer prognosis and a more advanced stage of the disease It may be concluded therefore that the prognosis may still be improved by earlier diagnosis, considering the excellent prognosis in the less advanced cases

The TNM system used in the analysis proved to be most useful, in accordance with the findings of others (SMITH et coll 1961, MORRISON & DEELEY 1962, JOHNSON & SISSON 1964, MCNELIS 1964, ALEXANDER CASSADY 1966, CAULK 1966, HEISE & BAYLIS 1966, RYGARD & HANSEN 1966, BACLESSE 1967, MARTENSON et coll 1967, SPENCER 1967, and TASKINEN 1969) The possibility of comparing the different series and of discussing the way of treatment has been greatly improved

The total result of a 63 % crude 5 year survival rate in our series is high compared with others published in recent years However, our series comprises mainly growths that were relatively moderately advanced, there being, in particular, but few T4 and N1 + 2 + 3 cases compared with other series These cases made up 14 % of the entire series Other materials, all of which are American except that of MARTENSON et coll (1967), have been of the following composition (the percentages stated represent the number of T4 and N1 + 2 + 3 cases in relation to the total cases)

ALEXANDER	~ 20 %	~ 60/306 cases
HEISE & BAYLIS	29 %	226/788
JOHNSON & SISSON	29 %	29/100
MCNELIS	42 %	39/93
MARTENSON et coll	15 %	87/578
SMITH et coll	33 %	201/600
Present series	14 %	35/248

None of the series appears to have been selected except the series of JOHNSON & SISSON and of MCNELIS It is thus evident that our series contained a relatively small number of advanced cases and a relatively large number of small growths

The decrement curves (Figs 2 and 3) representing the deaths from carcinoma

indicate that these occurred mainly within the first two years The practical consequences must be that materials analysed in this way afford an accurate expression of the efficacy of treatment in a series provided a reasonable number of cases have been followed for more than 3 years The special factors applying to the three main groups will now be discussed

The treatment in the supraglottic group is complicated mainly by the high incidence of lymph node metastases Dissection of the cervical lymph nodes with histologic evidence of lymph node metastases in nine cases did not lead to any cure whereas four cases with presumed metastases were cured by irradiation The tendency in recent years in centres using surgery as primary treatment seems to have been cervical node dissection in dealing with supraglottic carcinoma even when no lymph nodes are palpable but paying regard to the extent of the tumour (JACKSON & NORRIS 1962 McCOMB 1966)

The results within the group of glottic T1 cases are in the same range as reported from elsewhere (McCABE et coll 1960 CHABAZIAN et coll 1967 PEREZ 1968) An attitude to treat this group primarily by irradiation appears to exist in most centres

The glottic T2 cases may be classified as already mentioned into two subgroups with or without fixation of the vocal cords and these subgroups presented a distinct prognostic difference (59 % and 71 % crude 5 year survival rates respectively) HEISE & BAYLIS, dealing with the same problem reported a similar prognostic difference between the two subgroups 57 % for fixed and 70 % for unfixed cases On this basis it would seem reasonable to suggest that the T2 group be divided into two subgroups T2 + fix with fixation of cord(s) and T2 — fix without fixation of cord(s) It is tempting to transfer T2 + fix to the T3 group but probably not permissible since all T2 + fix cases cannot be said with certainty to be more extensive or more deeply infiltrating than T2 — fix cases

Another factor is the prognostic role of fixed vocal cords in all glottic carcinomas considered together The survival rates for the two groups with fixed and unfixed vocal cords in this series were 52 % and 76 % respectively This finding is not surprising considering that fixation is presumably usually a sign of more deeply infiltrating processes The prognostic role of vocal cord fixation has been discussed by SVANE KNUDSEN (1960), RYGARD & HANSEN (1966) and MARTENSON et coll (1967)

Glottic T3 cases from our series appear to be comparable with similar cases treated in other centres where surgery is the primary therapeutic principle The results are largely the same with crude 5 year survival rates of a little below 60 % The frequency of total laryngectomy in the surgical materials is about 85 % (SMITH et coll 1961) while in our series it was 43 % (23/53) Of the

5 year survivors 30 % (9/30) had undergone total laryngectomy Apart from that, a few cases in the T3 group (four in all) had had partial laryngectomy

The analysis of the glottic T3 group thus displayed the advantages of primary radiotherapy possibly followed by secondary surgery The survival rates are equal to those after primary surgery but the percentage of cases with preserved laryngeal function is about twice to three times higher than after primary surgery

The subglottic group comprised 13 cases Radiotherapy alone appears unsuitable for curing carcinoma in this location, whereas surgery, with or without irradiation, affords essentially better results, as suggested by FALBE HANSEN (1955) and MARTENSON et coll (1967) In advanced cases of subglottic carcinoma, meaning almost invariably carcinoma that has invaded the vocal cords and fixed them, the treatment of choice is perhaps complete radiotherapy followed by total laryngectomy and possibly dissection of cervical lymph nodes This rigorous procedure may be considered reasonable as there is a great risk of fatal delay in the detection of malignant residues or recurrence Possibly, it is also preferable in advanced laryngeal carcinoma in sites other than the subglottic, a view held by JOHNSON & SISSON (1964) GOLDMAN et coll (1968) have used this combined treatment in eight T4N0M0 cases, with a 100 % survival so far for 1 to 6 years

Conclusion

The results of treating laryngeal carcinoma mainly by primary irradiation must, in keeping with previous findings, be acceptable (NIELSEN 1954, CANTRIL 1960, TUDWAY & FREUNDLICH 1960, LEDERMAN 1961, WILSON 1961, BRYCE 1963 et coll , WANG & SCHULTZ 1963, ORMEROD 1964, BOZZI et coll 1966 JOLLES 1966, RYCARD & HANSEN 1966)

This therapeutic method affords results equal to those of primary surgery but unlike the latter possesses the important advantage of considerably increasing the number of patients with preserved laryngeal function Surgical series from recent years with excellent survival rates have had a laryngectomy frequency of 60 to 75 % (SMITH et coll 1961, JOHNSON & SISSON 1964, MCNELIS 1965, ALEXANDER & CASSIDY 1966, SPENCER 1967) By way of comparison it may be mentioned that the frequency in our series was 30 % (75/248) It must be borne in mind however that the series named in this comparison contained a relatively larger number of advanced cases Not uncommonly the view is maintained that the patient s chances of survival are reduced when radiotherapy rather than surgery is the primary treatment This view has been disproved by our results Another argument against primary radiotherapy has been that it prevents the possibility of subsequent supplementary minor surgical procedures (partial

laryngectomy) However it is apparent from the present results (Table 9) as well as those of others (FALBE HANSEN & SVANE KNUDSEN 1964) that partial laryngectomy may prove an excellent operation in spite of preceding irradiation

The safety of irradiation as the primary treatment is clearly apparent from the analysis presented in Table 8 A failure in radiotherapy irrespective of the cause does not necessarily affect the end result An effective follow up with early diagnosis of malignant residues or recurrence followed by secondary surgery, may bring the result almost up to the level where radiotherapy is at its best although of course at the cost of laryngeal function

SUMMARY

A total of 248 patients with carcinoma of the larynx were treated during the period 1944—1963 219 by primary irradiation and 29 by surgery The crude 5 year survival rate for the entire series was 63 per cent The 5 year survival rates with radiation therapy were largely the same as those obtained by surgery The number of patients with preserved laryngeal function was however twice to three times greater after treatment mainly by primary irradiation

ZUSAMMENFASSUNG

Insgesamt wurden 248 Patienten mit Kehlkopfkarzinom wahrend den Jahren 1944—1963 behandelt 219 Patienten wurden primar mit Bestrahlung behandelt und 29 Patienten wurden operiert Nach 5 Jahren waren 63 Prozent der Patienten am Leben Die 5 jahrige Überlebensrate fur die bestrahlten und die operierten Patienten war praktisch gleich Die Anzahl von Patienten mit erhaltener Kehlkopffunktion war jedoch zwei bis dreimal grosser nach Behandlung hauptsachlich mit Primarbestrahlung

RÉSUMÉ

Un total de 248 malades ont ete traites entre 1944 et 1963 pour cancer du larynx 219 malades ont été traités par irradiation primitive et 29 par chirurgie Le taux brut de survie à 5 ans pour la serie entiere est de 63 pour cent Le taux de survie a 5 ans apres radiotherapie est en gros le meme qu apres chirurgie Cependant le nombre de malades qui avaient conserve la fonction laryngee etait deux à trois fois plus eleve apres radiotherapie primitive

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CONCENTRATION OF ZINC IN SOME HARD AND SOFT TISSUES OF RAT DETERMINED BY NEUTRON ACTIVATION ANALYSIS

by

Bo Bergman

Zinc is an essential element for the organism and is present in most organs and tissues in man and animals in varying quantities. High concentrations have been reported for hard tissues, such as bone (Lutz 1926, Taylor 1961, Alexander & Nusbaum 1962, Soremark & Bergman 1962) and teeth (Cruickshank 1936, Samsahl & Soremark 1961, Lundberg et coll. 1965, Babicky & Taylor 1966, Nixon et coll. 1967). Previously reported values of zinc concentration in skeletal tissues have most often been concerned with long bones. High concentrations of zinc have been localized by means of autoradiography in mineralizing areas in long bones (Haumont & Vincent 1961, Haumont 1963, Kinnamon 1963).

The present study was initiated in order to compare quantitatively the zinc concentration of the following skeletal tissues of young and adult rats: mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. To allow for a more complete comparison with previous studies, the teeth and some soft tissues were also included. The results obtained in the present investigation are intended

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Table 1

Sensitivity of neutron activation analysis for zinc a compilation of values reported in the literature

Author(s)	Isotope measured	Neutron fluence cm sec	Irradiation time	Sensitivity
MEINKE 1955		5×10 10	Saturation	0 04 μg/ml 0 002 μg/ml
LEDDICOTTE et coll 1958	Zn	5×10	Saturation	0 02 ppm
BOWEN 1959	mZn	10	12 hours	0 05 ppm
PARR & TAYLOR 1964	Zn	10	7 days	0 01 ppm

to serve as a complement to a scintillation study on the distribution of radiozinc in rats (BERGMAN 1970)

Various methods have been utilized for the quantitative determination of micro elements in biologic materials For the determination of zinc in tissues and body fluids colorimetric methods have been used extensively (VALLEE & GIBSON 1948 BERFENSTAM 1952 WOLFF 1956 MALMSTROM 1956) Spectrographic analysis has been employed by SCOULAR (1939) GRIFFITH et coll (1954) MONACELLI et coll (1956) BUTT et coll (1964) PFEILSTICKER (1965) and STRAIN et coll (1966) and radiometric titration by LANDGREBE et coll (1968) Roentgen ray fluorescence has been used by ALEXANDER & NUSBAUM (1962) and ZEITZ & LEE (1966) and atomic absorption spectrophotometry by PRASAD et coll (1965), MACAPINLAC et coll (1966) PIHL & GUSTAFSON (1967) HACKLEY et coll (1968) and BOQUIST & LERNMARK (1969)

In recent years neutron activation has come into use more and more for analysis of microelements in biologic tissues The method has been reviewed and discussed in detail by e g BOWEN & GIBBONS (1963) SOREMARK (1965) and BOWEN (1966)

Values reported in the literature on the sensitivity of neutron activation analysis for zinc are compiled in Table 1 The sensitivity will be further increased by radiochemical separation of the radioactive zinc present in the sample (SAMSAHL 1961)

In the present study neutron activation analysis was chosen for the following reasons (SOREMARK 1965 BOWEN 1966) (1) there is a high degree of sensitivity and specificity and (2) the risk of contamination and loss can be almost completely eliminated

Material and Methods The animals used in the present study were albino rats (Sprague—Dawley) All animals were normally lively and no signs of infection

deficiency, or any disease were observed. Only females were used, there were ten 3 week old and ten 24 week old rats. The young animals were housed together with their mothers in acrylic cages with steel covers until sacrifice. The mothers of the young rats and the 24 week old rats had free access to tap water and a pellet rat diet, Anticimex 210 (Anticimex, Norrviken, Stockholm) with a zinc content of about 73 ppm.

The rats were mildly anaesthetized with ether and killed by decapitation. About 0.8 ml of blood was collected from each rat immediately after decapitation. The various tissues to be analysed were then rapidly excised in the following order: kidney, pancreas, spleen, liver (median lobe), heart, incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. The tissues were not perfused. When possible, the whole organ was removed. The teeth examined were the four incisors, which were broken off at the gingival margin, thus, only the crowns were analysed. All visible pulp was removed. The mandibular bone sample consisted of the ramus and part of the corpus including both spongy and compact bone. The periosteum was scraped away. When removing the tibia diaphysis, great care was taken to avoid the metaphyseal part. The diaphyseal part was dissected free, sectioned along its long axis, and freed of its bone marrow and periosteum. In this way, attempts were made to collect only the compact bone of the diaphysis. During the removal and preparation of the various specimens, no metal instruments were used. In order to avoid contamination only polyethylene or polyethylene covered instruments were in direct contact with the specimen. The specimens were collected in polyethylene tubes, and the wet weight was recorded immediately after removal of the tissue. The wet weights of the tissues varied between about 800 mg, e.g. blood and liver, and 15 mg, e.g. incisors and mandibular condyle. After weighing the specimens were dried in an electric oven for about 24 hours at 70 to 80° C in order to prevent volatilization during irradiation. Care was taken to avoid contamination of samples and standards before irradiation. The water used for dissolution of the standards to be sent for irradiation as well as that used for rinsing instruments and glassware was carefully purified according to the method described by SÖREMARK & JOHANSSON (1963) and collected in polyethylene bottles.

A standard amount of zinc was inserted into a separate polyethylene tube and placed in the same aluminium can as the specimens. The aluminium can containing the standard and the specimens was irradiated by thermal neutrons for periods of 5 and 12 days in the R2 reactor of the Swedish Atomic Energy Company in Studsvik, Nyköping. The neutron fluence was approximately 1.4×10^{1} cm sec^{-1}.

When the period of irradiation was completed the samples were dissolved in hot H_2SO_4 p.a. followed by dropwise addition of 30 % H_2O_2 p.a. Thereafter,

Table 2

Concentration of zinc in some hard and soft tissues from 3-week-old female rats — The values are based on wet weights and expressed in ppm $\bar{x}$ = mean of 10 rats s = standard deviation

Tissue	$\bar{x}$	s	s% = 100 s/$\bar{x}$
Blood	6 3	1 1	17
Kidney	18 6	2 9	16
Pancreas	30 0	5 7	19
Spleen	16 8	3 5	21
Liver	[illegible]3 0	18 3	35
Heart	14 6	4 3	29
Incisors	1[illegible]8 0	40 7	3[illegible]
Mandibular condyle	152 0*	57 7	38
Mandibular bone	1[illegible]1 0	25 0	21
Tibia epiphysis	45 0	9 5	21
Tibia diaphysis	140 0	22 8	16

* One sample spoiled during preparation mean value based on nine samples

Table 3

Concentration of zinc in some hard and soft tissues from 24 week old female rats — The values are based on wet weights and expressed in ppm $\bar{x}$ = mean of 10 rats s = standard deviation

Tissue	$\bar{x}$	s	s% = 100 s/$\bar{x}$
Blood	13 0	15 4	118
Kidney	18 4	4 2	[illegible]3
Pancreas	2[illegible] 6	19 8	88
Spleen	63 7	28 1	44
Liver	44 9	33 4	74
Heart	14 5	3 4	23
Incisors	111 0	33 0	30
Mandibular condyle	223 0	84 9	38
Mandibular bone	[illegible]04 0	61 0	30
Tibia epiphysis	250 0	5 5	21
Tibia diaphysis	250 0	[illegible]1 9	[illegible]1

the solution was diluted with 0 7 n HCl p a The diluted solution flowed at a rate of 2 ml/min through an anion exchange column in chloride form Dowex 2 × 10 The columns used were 10 cm high with a diameter of 1 cm A faint suction was applied by means of a vacuum pump After the dropping had ceased the column was washed with 50 ml of 0 7 n HCl p a in order to remove traces

Table 4

Some previous results on zinc concentration in various rat tissues compiled together with the results obtained in the present study — Mean values are given and the concentrations are expressed in ppm

Year	Author	Rat age in weeks	Method
1926	LUTZ		Fluorescence
1927	HELLER & BURKE		Fluorescence
1938	HOVE et coll		Colorimetry
1940	DAY & McCOLLUM	15	Colorimetry
1951	MAWSON & FISCHER		Colorimetry
1956	GILBERT & TAYLOR	6—12	Colorimetry
1958	MILLAR et coll	12—13	Colorimetry
1960	FORBES & YOHE		Colorimetry
1961	FORBES	9	Colorimetry
1961	TAYLOR	8—49	Colorimetry
1962	ALEXANDER & NUSBAUM	0—59	Roentgen ray fluorescence and emission spectrography
1966	HUXLEY & LEAVER	14—15	Polarography
1966	MACAPINLAC et coll	10	Atomic absorption spectrophotometry
1967	REINHOLD et coll		Colorimetry
1967	PRASAD et coll	3	Atomic absorption spectrophotometry
1968	SWENERTON & HURLEY		Atomic absorption spectrophotometry
Present study		3	Neutron activation analysis
		24	Neutron activation analysis

of ^{24}Na. The standards were treated in exactly the same way as the biologic samples. The radiochemical separation method used was a modification of the one described by SAMSAHL et coll. (1963).

After the radioactive zinc had been collected in the anion exchange column, the resin was transferred to stoppered polyethylene tubes for analysis in a 5″ × 3″ well type NaI (Tl) scintillation detector connected to a transistorized 512 channel gamma spectrometer.

Quantitative data based on the wet weight of the organ samples were obtained by comparing the gamma intensity, the photopeak area at 1.1156 MeV of ^{65}Zn

Table 4 *(cont)*

Weight	Whole blood	Kidney	Pancreas	Spleen	Liver	Heart	Teeth	Bone
Wet	6 7	14 4		36 3	20 7			178 4
Wet		15		31	20	14		
Dry					76		9? (dent n and enamel)	92
Ash								237 233 (tibia)
Wet			23 3		30 3			
Wet	3 88	23 4		?4	29 7			133 9
Wet					43			
Wet								285 (femur)
Ash								77—191 (femur)
Wet								
Ash								323 (femur shaft) 420 (femur end)
Dry							2?0 (dentin) 130 (e amel)	388 (femur)
Wet		41			34			162 (femur)
Wet		19 4	17 ?	16 ?	?3 9			
Dry		91		10?	101	73		168
Ash								424 (femur)
Wet	6 3	18 6	30	16 8	53	14 6	128 (dent n and enamel)	140 (tibia diaphysis)
Wet	13	18 4	? 6	63 7	44 9	14 5	111 (dent n and enam l)	2?0 (t bia diaphysis)

or at 0 439 MeV of ^{69m}Zn in the sample with that of the standard (MARINELLI et coll 1952)

The method used has been discussed by SAMSAHL & SOREMARK (1961) SOREMARK & BERGMAN (1962) SAMSAHL *et coll* (1963) BOWEN & GIBBONS (1963) OLEHY et coll (1966) and HAHN *et coll* (1968) BRUNE (1963) reported a yield for ^{65}Zn of 98 % with a standard deviation of 8 % by the chemical group separation method developed by SAMSAHL et coll (1963) The loss of zinc due to the chemical procedures after the irradiation was also tested in the present study To each of four hard and four soft tissue samples was added a known amount of ^{65}Zn The samples then underwent the same chemical procedure as

the irradiated samples and were subjected to scintillation measurements The yield was 97 3 % with a standard deviation of 3 5 %

Various errors in the sampling method have been analysed elsewhere (BERGMAN 1970), and were found to be small Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24 week old rats — can be ascribed mostly to biological variations

Results

The zinc concentrations obtained by means of neutron activation and gamma ray spectrometric analysis of some hard and soft tissues from 3- and 24 week old rats are presented in Tables 2 and 3

Intra and interindividual differences were tested by means of Student s t test Except for tibia epiphysis in the 3 week animals, all the bone samples ($p < 0\,001$) and the incisors ($p < 0\,01$), had significantly higher zinc concentrations than the soft tissue samples within both age groups For incisors, blood, kidney, pancreas, liver, and heart, there were no significant differences between the two age groups The zinc concentration was significantly higher in adult rats in spleen ($p < 0\,001$), mandibular bone ($p < 0\,01$), tibia epiphysis ($p < 0\,001$) and tibia diaphysis ($p < 0\,001$) For mandibular condyle the zinc concentration was almost significantly higher in adult rats ($p \sim 0\,05$)

Discussion

Comparisons of different studies concerning microelements such as zinc in biologic tissues are complicated for many reasons The materials and methods vary and results are expressed in different ways, e g based on wet weight, dry weight, or ash weight Furthermore, age is an important variable for the zinc concentration in skeletal samples

For all the tissues analysed in the present study, except mandibular condyle and mandibular bone data on zinc concentrations in rats are available from earlier studies In Table 4, some previously reported results for rat tissues are compiled together with the results obtained in the present study

Where comparisons can be made, the results obtained in the present study agree comparatively well with those previously published Spleen was the only soft tissue tested where a significant difference could be shown between the two age groups in the present study At present no explanation can be given for this difference It is also noteworthy that the zinc concentration in the spleen of the 24 week old rats differs considerably from previously published values (Table 4)

It was found in the present study that the skeletal samples of the adult rats

Table 5

Concentration of zinc in some hard tissues of female rats — The values in Tables 2 and 3 have been used and are here recalculated on ash weight and expressed in ppm — Numbers within parentheses indicate the mean ratios ash wet ht/wet weight each obtained from 6 rats $\bar{x}$ = mean of 10 rats s = standard deviation

Tissue	3 week rats		24 week rats	
	$\bar{x}$	s	$\bar{x}$	s
Incisors	197 (65)	63	154 (72)	46
Mandibular condyle	755 (20)	242	572 (39)	218
Mandibular bone	404 (30)	83	371 (55)	111
Tibia epiphysis	566 (8)	188	735 (34)	154
Tibia diaphysis	350 (40)	46	391 (64)	81

had a significantly higher zinc concentration per gram wet weight than those of the young rats for mandibular condyle the difference was almost significant The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962) TAYLOR (1961) reported that the mean zinc content of femur humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight The zinc content of the ribs did not increase as much as that of the other bones ALEXANDER & NUSBAUM (1962) contrary to TAYLOR (1961) were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days These authors were also unable to show any correlation between age and zinc content in human ribs They found it likely that the level of zinc in bone was directly *related* to the zinc to calcium ratio of the diet The results of TAYLOR (1961) and those of the present study are based on wet weight while those of ALEXANDER & NUSBAUM (1962) are based on ash weight This fact complicates *direct* comparisons between the studies The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

the irradiated samples and were subjected to scintillation measurements The yield was 97 3 % with a standard deviation of 3 5 %

Various errors in the sampling method have been analysed elsewhere (BERGMAN 1970), and were found to be small Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24 week old rats — can be ascribed mostly to biological variations

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Tibia epiphysis	566 (8)	188	735 (34)	154
Tibia diaphysis	350 (40)	46	391 (64)	81

had a significantly higher zinc concentration per gram wet weight than those of the young rats for mandibular condyle the difference was almost significant The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962) TAYLOR (1961) reported that the mean zinc content of femur humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight The zinc content of the ribs did not increase as much as that of the other bones ALEXANDER & NUSBAUM (1962) contrary to TAYLOR (1961) were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days These authors were also unable to show any correlation between age and zinc content in human ribs They found it likely that the level of zinc in bone was directly related to the zinc to calcium ratio of the diet The results of TAYLOR (1961) and those of the present study are based on wet weight while those of ALEXANDER & NUSBAUM (1962) are based on ash weight This fact complicates direct comparisons between the studies The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

the irradiated samples and were subjected to scintillation measurements The yield was 97 3 % with a standard deviation of 3 5 %

Various errors in the sampling method have been analysed elsewhere (BERGMAN 1970), and were found to be small Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24 week old rats — can be ascribed mostly to biological variations

Results

The zinc concentrations obtained by means of neutron activation and gamma ray spectrometric analysis of some hard and soft tissues from 3 and 24 week old rats are presented in Tables 2 and 3

Intra and interindividual differences were tested by means of Student's t test Except for tibia epiphysis in the 3 week animals, all the bone samples ($p < 0.001$) and the incisors ($p < 0.01$), had significantly higher zinc concentrations than the soft tissue samples within both age groups For incisors, blood, kidney, pancreas liver, and heart, there were no significant differences between the two age groups The zinc concentration was significantly higher in adult rats in spleen ($p < 0.001$), mandibular bone ($p < 0.01$), tibia epiphysis ($p < 0.001$), and tibia diaphysis ($p < 0.001$) For mandibular condyle the zinc concentration was almost significantly higher in adult rats ($p \sim 0.05$)

Discussion

Comparisons of different studies concerning microelements such as zinc in biologic tissues are complicated for many reasons The materials and methods vary and results are expressed in different ways e g based on wet weight, dry weight, or ash weight Furthermore, age is an important variable for the zinc concentration in skeletal samples

For all the tissues analysed in the present study, except mandibular condyle and mandibular bone data on zinc concentrations in rats are available from earlier studies In Table 4 some previously reported results for rat tissues are compiled together with the results obtained in the present study

Where comparisons can be made, the results obtained in the present study agree comparatively well with those previously published Spleen was the only soft tissue tested where a significant difference could be shown between the two age groups in the present study At present no explanation can be given for this difference It is also noteworthy that the zinc concentration in the spleen of the 24 week old rats differs considerably from previously published values (Table 4)

It was found in the present study that the skeletal samples of the adult rats

Table 5

Concentration of zinc in some hard tissues of female rats — The values in Tables 2 and 3 have been used and are here recalculated on ash weight and expressed in ppm — Numbers within parentheses indicate the mean ratios ash weight/wet weight each obtained from 6 rats $\bar{x}$ = mean of 10 rats s = standard deviation

Tissue	3 week rats			24 week rats		
	$\bar{x}$		s	$\bar{x}$		s
Incisors	197		63	154		46
		(65)			(72)	
Mandibular condyle	755		242	572		218
		(20)			(39)	
Mandibular bone	404		83	371		111
		(30)			(55)	
Tibia epiphysis	566		188	735		154
		(8)			(34)	
Tibia diaphysis	350		40	391		81
		(40)			(64)	

had a significantly higher zinc concentration per gram wet weight than those of the young rats for mandibular condyle the difference was almost significant The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962) TAYLOR (1961) reported that the mean zinc content of femur humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight The zinc content of the ribs did not increase as much as that of the other bones ALEXANDER & NUSBAUM (1962) contrary to TAYLOR (1961) were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days These authors were also unable to show any correlation between age and zinc content in human ribs They found it likely that the level of zinc in bone was directly related to the zinc to calcium ratio of the diet The results of TAYLOR (1961) and those of the present study are based on wet weight while those of ALEXANDER & NUSBAUM (1962) are based on ash weight This fact complicates direct comparisons between the studies The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

those based on ash weight. Six 3 week old and six 24 week old rats were injected intraperitoneally with 0.2 μCi ^{65}Zn per gram body weight and killed after 24 hours. The tibia diaphysis of the hind legs of each rat was dissected free and freed from bone marrow and periosteum, the wet weight was recorded, and the total concentration of ^{65}Zn in the bone samples determined. The ash weight was recorded after 16 hours at 600° C (ad modum ROBINSON & ELLIOT 1957, ALEXANDER & NUSBAUM 1962), and the total concentration of ^{65}Zn was determined in the ashed samples. By using metabolically incorporated ^{65}Zn in this way, it could be verified that no loss of zinc took place during the conditions of ashing. The ratio ash weight/wet weight was further recorded for the incisors, mandibular condyle, mandibular bone and tibia epiphysis. Using these figures, the zinc concentrations for the hard tissues (Tables 2 and 3) were recalculated on the basis of ash weights (Table 5). When the differences between 3 and 24 week old rats were tested statistically, only the mean ratios ash weight/wet weight were considered, as the standard deviations of these mean ratios were of negligible magnitude (< 3 %).

No significant differences could be found between the young and adult rats using the ash weights for the zinc concentrations in the incisors, mandibular condyle, mandibular bone or tibia diaphysis. For tibia epiphysis, the adult rats showed an almost significantly higher zinc concentration than the young rats ($p < 0.05$). The mean values for zinc concentration in ashed tibia diaphysis in the present study (350 and 391 ppm) agree well with those reported by MAWSON & FISCHER (1951) for ashed tibia (390 ppm = 233 ppm wet weight) and ALEXANDER & NUSBAUM (1962) for ashed femur shaft (523 ppm). Thus, it appears that zinc concentration in skeletal tissues and incisors of rats increases as mineralization proceeds, corresponding to the situation in the human enamel. Surface enamel in man has been reported to contain a higher concentration of mineral than the deeper layers (THEWLIS 1940, SONI & BRUDEVOLD 1959, ANGMAR et coll. 1963), and BRUDEVOLD et coll. (1963) found zinc to be more concentrated in surface layers than in deeper layers of the enamel in man. The most striking difference obtained in the present study between young and adult rats was found for tibia epiphysis: 45 and 250 ppm zinc based on wet weights. This difference may reflect the late start in mineralization as indicated by the low ash weight at three weeks and the continuing replacement of existing epiphyseal cartilage with bone with increasing age.

No significant difference could be found in the present study between the incisors in the two age groups. This can probably be explained by the fact that the rat incisors are almost fully mineralized at 3 weeks of age as indicated by the ash weight/wet weight ratios, and thereafter are subject to continuous growth and abrasion.

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SUMMARY

The concentration of zinc (ppm/wet weight) was determined in the mandibular condyle and in some selected hard and soft tissues of 3- and 24-week old rats by means of neutron activation and gamma ray spectrometry. Except for tibia epiphysis in the 3-week old animals the skeletal samples and the incisors had significantly higher zinc concentration than the soft tissue samples in both age groups. Excepting the spleen, which had a significantly increased zinc concentration in adult rats, none of the soft tissues sampled showed significant differences in zinc concentrations between young and old rats. The skeletal samples of the adult rats had a significantly higher zinc concentration than those of the young rats. These increases were shown to result most probably from increasing mineralization with increasing age. No significant difference was found between the zinc concentrations of the incisors of the two age groups.

ZUSAMMENFASSUNG

Die Konzentration von Zink (ppm/Feuchtgewicht) wurde in der Mandibularcondyle und verschiedenen ausgewählten harten und weichen Geweben von 3 und 24 Wochen alten Ratten mit Hilfe von Neutronenaktivierung und Gammastrahlen-Spektrometrie bestimmt. Mit Ausnahme der Epiphyse der Tibia von 3 Wochen alten Tieren war die Zink-Konzentration der Skelettproben und der Inzisoren in beiden Altersgruppen signifikant höher als in den Proben der weichen Gewebe. Mit Ausnahme der Milz, deren Zink-Konzentration bei erwachsenen Ratten signifikant angestiegen war, bestanden keine signifikanten Unterschiede der Zink-Konzentration der Proben weicher Gewebe zwischen jungen und alten Ratten. Die Skelettproben erwachsener Ratten hatten eine signifikant höhere Zink-Konzentration als die der jungen Ratten. Dieser Anstieg ist am wahrscheinlichsten das Resultat der steigenden Mineralisation mit steigendem Alter. Es wurde keine signifikante Differenz zwischen der Zink-Konzentration der Inzisoren der beiden Altersgruppen gefunden.

RÉSUMÉ

L'auteur a déterminé par activation neutronique et par spectrométrie de rayons gamma la teneur en zinc (ppm/poids humide) dans le condyle mandibulaire et dans certains tissus durs et mous de rats agés de 3 et de 24 semaines. A l'exception de l'épiphyse tibiale des rats agés de 3 semaines, les échantillons osseux et les incisives avaient une teneur en zinc significativement plus élevée que celle des échantillons de tissu mou dans les deux groupes d'age. A l'exception de la rate dont la teneur en zinc était significativement augmentée chez les rats adultes, aucun des échantillons de tissu mou ne présentait de différence significative de leur teneur en zinc entre les jeunes rats et les vieux rats. Les échantillons osseux de rats adultes avaient une teneur en zinc significativement plus élevée que celle de jeunes rats

L'auteur a montré que cette élévation de la teneur en zinc résulte très probablement de l'augmentation de la minéralisation osseuse en fonction de l'âge. Il n'a pas constaté de différence significative de la teneur en zinc des incisives dans les deux groupes d'âge.

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RADIOPATHOLOGY OF AMERICIUM 241

I. Distribution of americium in adult mice

by

LARS HAMMARSTRÖM and AGNAR NILSSON

The increasing production and use of transuranic elements have enhanced the interest in their relative toxicities, metabolic behaviour and carcinogenic properties. At present the maximum permissible levels for transuranic elements are based on the assumption that their biologic risks are similar to those of plutonium; however, this does not always seem to be true. A comparison of the uptake of ^{241}Am and ^{239}Pu in the skeleton of rats indicated that the initial uptake of ^{239}Pu in the skeleton was about 1.4 times greater than that of ^{241}Am, while the rate of loss from the skeleton was almost the same for these nuclides. The rate of excretion from the body was higher for ^{241}Am than for ^{239}Pu and the retention of the latter in the liver was of a longer duration than that of ^{241}Am (TAYLOR et coll. 1961).

Differences also exist in terms of biologic effects. BENSTED et coll. (1965) have proved that 77 % (17/22) of rats given 3.0 μCi ^{239}Pu/kg body weight developed bone tumours. In addition, one case of renal carcinoma and one of myelogenous leukemia were observed. After the injection of 2.5 μCi ^{241}Am/kg body weight

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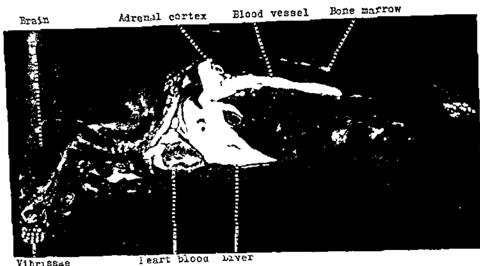


Fig 2 Autoradiogram Distribution of ^{241}Am in a male mouse 30 minutes after intravenous injection High concentration in the liver and adrenal cortex the concentration in the blood is lower than in the liver no demonstrable radioactivity in the bone but a moderate concentration in the marrow

freezing in hexane cooled with solid CO_2 ($-70°$ C) One male mouse was killed at each of the time intervals of 5 minutes 30 minutes 24 hours 4 days and 15 days after injection Two mice were killed 30 days after injection and two after 60 days The female mice that were injected on the 15th day of gestation were killed 24 hours and 4 days after injection and the two that were injected on the 18th day of pregnancy were killed 4 hours and 24 hours after injection

The animals immediately before freezing were placed in an aqueous solution of carboxymethylcellulose applied on a large microtome stage The specimens after freezing in hexane CO_2 were thus ready for sectioning which was performed in a freezebox (-15 C) To obtain sections through the whole animals adhesive tape (No 810 Minnesota Mining and Manufacturing Co) was attached to the exposed surface of the frozen specimen before cutting the sections then came off adhering to the tape Sections 20 μ thick were taken and freeze dried in the box for 2 days after which they were brought to room temperature in an air tight box The sections were then pressed against a roentgen film (Structurix D7 Gevaert) and exposed for four weeks After the sections had been removed the films were developed in G 230 and fixed in D 303 (Gevaert) the sections were stained with hematoxylin and eosin The autoradiographic procedure has been described in detail by ULLBERG (1954 1958)

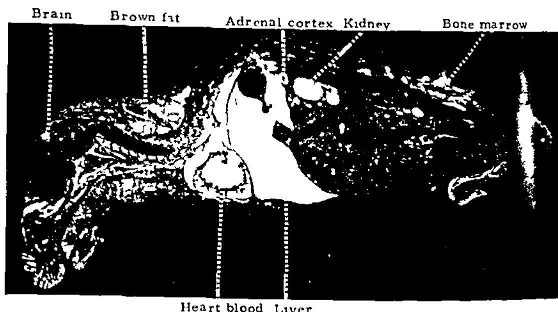


Fig 1 Autoradiogram Distribution of ^{241}Am in a male mouse 5 minutes after intravenous injection High concentration (light areas) in the blood and liver as well as in the adrenal cortex no demonstrable radioactivity in the bone but the marrow displays a moderate concentration

only four out of nineteen rats (21 %) developed osteosarcomas Adrenal tumours were detected in two animals and one animal developed leukemia of an undetermined type

As part of an investigation of the pathologic effects of ^{241}Am in mice the present study of the distribution of this nuclide was performed in order to obtain more information concerning the organs at risk

Material and Methods

Labelled compound Americium 241 with a concentration of 3 8 μCi/ml was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England The radioactive compound was administered as a nitrate

Animals Nine adult male mice and four pregnant female mice of the CBA strain were used Two of the female mice were injected on the 15th day of gestation and the other two female mice on the 18th day of gestation The average weight of the male mice was 20 g and of the pregnant mice 30 to 35 g

Autoradiographic procedure Each animal was given 0 26 ml of the solution of ^{241}Am, corresponding to 1 μCi intravenously in a tail vein After predetermined survival periods the animals were anaesthetized with ether and killed by

Bone There was a latency in the uptake of americium in the bone After 4 hours a deposition at the endosteal and periosteal surfaces of bone was observable and after 24 hours there was a high concentration at these sites The concentration at the endosteal surfaces was usually higher than at the periosteal surfaces The concentration as well as the localization seemed to be unchanged during the remaining investigation period The bone marrow had a moderate concentration of ^{241}Am at all the intervals studied

Cartilage Americium was taken up in the tracheal and auricular cartilage As in bone there was a latency of some hours in the uptake which was limited to the surface The intervertebral discs had no detectable amounts of the injected ^{241}Am

Teeth A marked accumulation was observed in the dental pulp and in the periodontal membrane (cf Fig 5) There seemed to be some radioactivity at the surface of the developing enamel of the incisors

Gastro intestinal tract Some radioactivity was seen in the contents of the stomach close to the secretory mucosa in all the animals studied and a small amount of radioactivity was also present in the intestinal lumen after long survival periods No radioactivity appeared in the gastric or intestinal mucosa however Five minutes after injection the concentration of ^{241}Am in the liver was the same as in the circulating blood It then seemed to increase slightly and at all the intervals studied was the highest in the body The distribution was fairly even hortly after injection but with time a redistribution towards a higher concentration around the central veins occurred Americium was never observed in the gall bladder (cf Fig 6) The salivary glands and the pancreas never had any ob ervable amounts of ^{241}Am

Respiratory tract The concentration in the lungs followed that of the circulating blood A high concentration was recorded in the bronchial cartilage however

Urinary tract The whole kidney exhibited a moderate concentration of radioactivity and in addition a higher accumulation was observed in small spots in the renal cortex No accumulation was present in the renal pelvis but radioactivity over the mucosa of the ureter and urethra persisted up to 60 days after the injection

Endocrine organs The adrenal cortex had a fairly high concentration which persisted 60 days after the injection at 5 minutes it had become evenly distributed Twenty four hours and more after the injection the concentration in an outer zone presumably the zona glomerulosa exceeded that of the other zones of the cortex This higher concentration in the outer zone was not present in the female mice No radioactivity was observed in the adrenal medulla A low concentration was present in the pituitary thyroid and pancreatic islets Some radio-

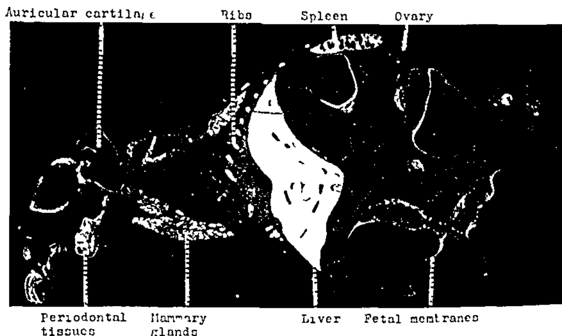


Fig 3 Autoradiogram Distribution of ^{241}Am in a pregnant mouse (19th day of gestation) 24 hours after intravenous injection Uptake in the liver endosteal and periosteal parts of the bone periodontal tissues auricular cartilage fetal membranes mammary glands and red pulp of the spleen

Results

Shortly after the intravenous injection of americium the highest concentration appeared in the blood and the liver Some other richly vascularized tissues, such as the bone marrow, spleen, kidney, adrenal cortex, lungs, brown fat and nasal mucosa had a moderate concentration No radioactivity was noted in the mineralized tissues, shortly after injection (Fig 1)

The concentration in the blood decreased during the first few hours and after 4 hours radioactivity was no longer discernible The liver and the skeletal tissues seemed to be the major sites of deposition A high and persistent uptake of radioactivity was also seen in the adrenal cortex, a few ovarian follicles, the marginal sinuses of the spleen, and the dental pulp The distribution pattern remained fairly unchanged during the whole investigation period

The distribution in the different tissues will be described more in detail below

The excretion of the injected americium appeared to occur slowly, only a slight decrease of radioactivity in the organism was autoradiographically observable 30 to 60 days after injection

Blood The concentration of americium was high shortly after injection but ... radioactivity was seen

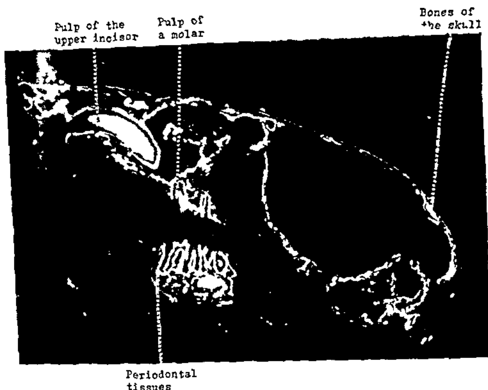


Fig 5 Autoradiogram Distribution in the head of a mouse 24 hours after intravenous injection of ^{241}Am High concentration in the bone periodontal tissues and dental pulp

activity in these glands seemed to persist when no radioactivity was demonstrable in the circulating blood

Gonads The uptake in the testes was low but appeared to increase moderately with time The radioactivity was mainly localized in the interstitial tissue The ovaries of the pregnant mice had a high concentration in some follicles and a moderate concentration in the interstitium while the corpora lutea displayed a lower concentration (Fig 4)

The central nervous system presented no evidence of radioactivity

Lymphatic tissues The red pulp of the spleen had a fairly high concentration which seemed to remain unchanged during the whole period of investigation The marginal sinuses had a higher concentration than the red pulp (Fig 4) No radioactivity was present in the white pulp of the spleen or in the lymph glands

Muscles The muscles had no detectable concentration

Skin and subcutaneous tissue The vibrissa follicles had an uptake of americium that gradually disappeared during the first day after the injection (Fig 2) N

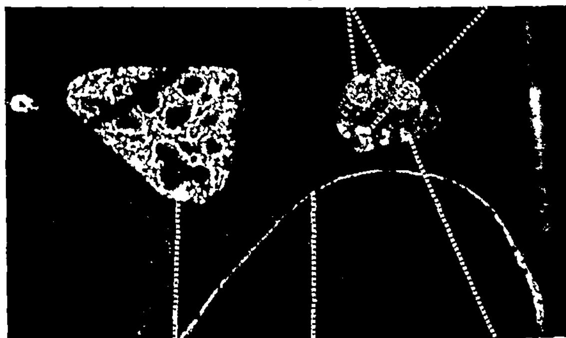




Fig 4 Detail of autoradiogram (upper image) of a pregnant mouse (15th day of gestation) 4 hours after intravenous injection of ^{241}Am and the corresponding stained section (lower image) depicting the distribution of ^{241}Am in the ovary spleen and adjacent placenta high uptake in some follicle walls of the ovary

owever a moderate concentration was noted in the fetal membranes (Figs 3 nd 4)

Mammary glands had a moderate concentration at all the intervals studied

Fetuses Only little radioactivity was seen in the fetuses and only a faint representation of the skeleton was obtained in the animals injected at the 18th ay of gestation

Discussion

The distribution of americium in the present investigation was predominantly characterized by an accumulation of the isotope in the bone and liver tissues This s in close agreement with results obtained earlier with the impulse counting echnique (SCOTT et coll 1945) and seems generally to be a common feature for all the actinide elements (TAYLOR 1964) The preferential accumulation of americium at the endosteal surfaces of bone appears to be compatible with previous findings that its concentration is higher on resting and resorbing surfaces of bone than on those where bone formation is in progress (TAYLOR et coll 1966 HERRING et coll 1962) The mechanism of binding of americium to bone tissue is not known although some evidence that it is bound to bone glycoproteins (HERRING et coll 1962 CHIPPERFIELD & TAYLOR 1968) exists The finding in the present investigation that there was a latency in the uptake of the isotope in bone after an intravenous injection may indicate that americium is incorporated into a larger molecule that has an affinity for skeletal tissues

Certain new sites of marked accumulation were also detected in the present investigation i e the adrenal cortex the ovary and the dental pulp The accumulation in the adrenal cortex may be placed in relation to the americium induced adrenal tumours in rats observed by BENSTED et coll (1965) However ^{241}Am is not taken up in the adrenal cortex of young rats (HAMMARSTROM & NILSSON to be published) This may indicate that the binding mechanism is in some way associated with steroid hormone production after sexual maturation Of other actinide elements studied both plutonium 239 (ULLBERG et coll 1962) and uranium 233 (WALINDER et coll 1965) have been reported to be accumulated in the adrenal cortex The relative concentration in the adrenal cortex seems however to be considerably less for these two radioelements Like americium also these two actinide were accumulated and retained in the ovary and the interstitial cells of the testes Local irradiation of the reproductive cells may have grave genetic consequence Our preliminary results concerning the pathologic effect of ^{241}Am have revealed serious atrophy of the testes

The accumulation in the dental pulp noted in this investigation does not seem to have been observed for other actinide elements The concentration

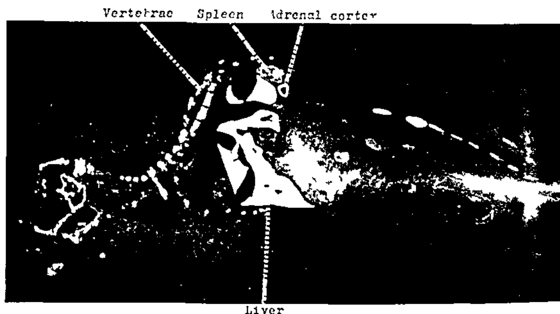


Fig 6 Autoradiogram Distribution of ^{241}Am in a mouse 1? days after intravenous injection Attention may be drawn to the outer zone of the adrenal cortex and the marginal sinuses of the spleen

uptake was noted in other hair follicles or in other parts of the skin or subcutaneous tissue

Brown fat A moderate concentration was seen in the brown fat The disappearance from this tissue was slower than from the blood

Placenta No radioactivity was present in the placenta at the intervals studied

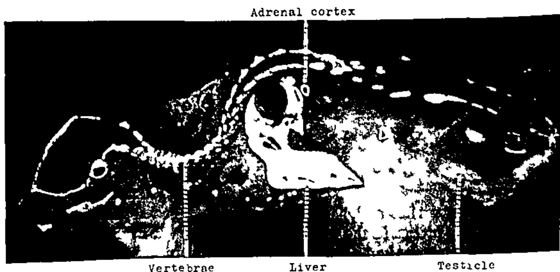


Fig 7 Autoradiogram Distribution of ^{241}Am in a mouse 60 days after intravenous injection Attention may be drawn to the adrenal cortex liver and bone

FROM THE DEPARTMENT OF ANIMAL GENETICS NUTRITION AND HYGIENE (DIRECTOR PROF. DYRENDAHL) THE ROYAL VETERINARY COLLEGE STOCKHOLM AND THE DIVISION OF RADIOBIOLOGY (DIRECTOR DOCENT A NILSSON) THE RESEARCH INSTITUTE OF NATIONAL DEFENCE SUNDBYBERG, SWEDEN

ROENTGEN RAY EFFECTS ON THE OVARIES OF FOETAL MICE

by

B HENRICSON and A NILSSON

The effect of external irradiation on the gonads of post natal female mice has been the subject of numerous investigations (OAKBERG 1958 1960 1962 1966 1968 PETERS 1961 PETERS & BORUM 1961 PETERS & LEVY 1964 RUSSELL & RUSSELL 1956 PARSONS 1962 and others) When exposing the foetal ovary of rats to roentgen rays (50 to 100 R) BEAUMONT (1962 1966) found an increasing sensitivity of the germ cells between the 8th and 15th days of foetal life In mice MINTZ (1959) revealed a very sensitive period on the 11th and 12th days of foetal life The dose given was 100 to 300 R

Concerning internal emitters NILSSON & HENRICSON (1969) have reported on the effect of 20 μCi ^{90}Sr per mouse given intravenously on the 11th or 16th day of intrauterine life The effect was clearly higher on the 16th day

The object of the present investigation was to study the effect of two doses of roentgen treatment (20 and 80 R) on the 11th day of uterine life and to compare it to the ^{90}Sr treatment

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appeared to be about the highest in the body and radiation injuries might well be expected It would appear that a primary tumour of the dental pulp has never been reported and special attention will be paid to this tissue in a current long time study of the radiopathology of americium 241

SUMMARY

The distribution and retention in mice of ^{241}Am after a single intravenous injection were investigated by autoradiography The technique is described and the findings are discussed in detail

ZUSAMMENFASSUNG

Die Verteilung und Aufspeicherung des ^{241}Am wurde mittels Autoradiographie nach einer einzelnen intravenosen Injektion studiert Die Technik der Methode und deren Resultate werden im einzelnen beschrieben

RÉSUMÉ

La repartition et la fixation du ^{241}Am chez les souris apres une injection intraveineuse unique ont eté étudiee par autoradiographie Les auteurs décrivent la technique et analysent en detail les resultats

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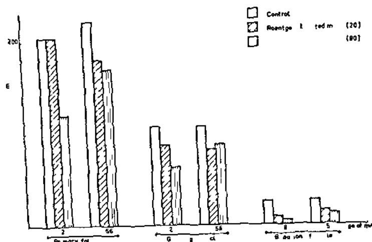


Fig 3 Average number of primary growing and Graafian follicles

The ovaries of the animals were immediately removed after sacrifice and fixed in Stieve's fluid The right ovary was serially sectioned at 5 μM Every tenth section was investigated after staining with Ehrlich's haematoxylin eosin The following types of oocytes and follicles were identified and counted

Oocytes group I Oocytes without follicular cells

Oocytes group II Oocytes with 1 to 4 follicular cells

Oocytes group III Oocytes with 5 follicular cells to an almost complete single layer of follicular epithelium

Primary follicles Oocytes surrounded by one complete layer of follicular cells

Growing follicles Two or more layers of follicular cells without complete formation of an antrum

Graafian follicles Complete antrum formation in the follicular epithelium

Atretic follicles Pyknotic and lytic cells in the follicular layers usually containing a shrunken oocyte with disintegration of zona pellucida

Corpus atreticum The visible end result of atresia a highly degenerated oocyte surrounded by a glass membrane and peripheral to it some theca cells and connective tissue

Early antrum Growing follicles with two layers of follicular cells the intercellular tissue in the stratum granulosum is loose and may represent the initiation of formation of large pools of follicular liquid characteristic of Graafian follicles

The total number of cells per ovary in each group of mice was calculated by using the formula of ABERCROMBIE (1946)

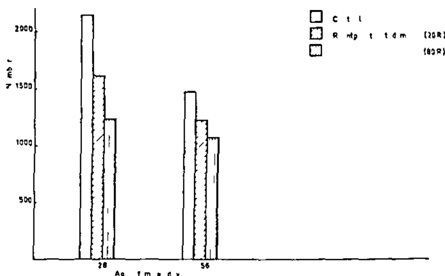


Fig 1 Total number of oocytes and follicles Average of five mice irradiated on the 11th day of foetal life and killed 28 or 56 days after birth

Material and Methods Inbred CBA mice were irradiated on the 11th day of foetal life The doses were 20 or 80 R The roentgen equipment was a Muller MG 300 apparatus operated at 260 kV, 10 mA, focal distance 45 cm, inherent filtration 4 mm Al, additional filter 0 5 mm Cu, giving a dose rate of 75 R/min Groups of five mice, including also unirradiated controls, were killed at 28 or 56 days after delivery

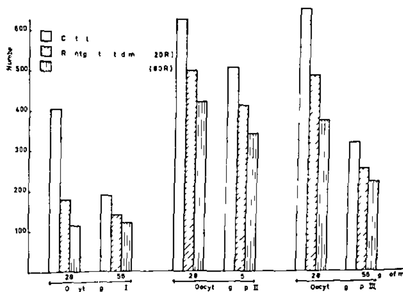


Fig 2 Average number of oocytes of different types

following 100 R In our experiments with mice the reduction of oocytes was about 25 % after 20 R and 45 % after 80 R when estimating the numbers 28 days after birth There is consequently some indication of higher sensitivity of primordial germ cells in the rat than in the mouse However in BEAUMONT s series the dose rate was about three times higher than in our experiment

When evaluating the number of oocytes and follicles at the age of 56 days however we found a smaller decrease in cells than at the age of 28 days The reduction at 56 days amounted to 19 % after 20 R and 28 % after 80 R There seems consequently to have been some repair between 28 and 56 days of age

In an earlier experiment (NILSSON & HENRICSON 1969) female mice were treated with a ^{90}Sr dose of 20 μCi per mouse on the 11th day of pregnancy The killing effect on primordial germ cells was far greater than with even the highest roentgen dose (80 R) used in the present experiment Following ^{90}Sr the reduction of oocytes and follicles at the age of 28 days was about 64 % and 56 days 58 %

SUMMARY

Female mice were irradiated on the eleventh day of pregnancy with roentgen radiation of 20 R or 80 R The female offspring were killed at the age of 28 or 56 days and oocytes and follicles were counted There was a clear dose related reduction of the oocytes amounting to between 20 and 45 % in comparison with the controls The fact that the reduction was less at the age of 56 days than at 28 days suggests a repairing process

ZUSAMMENFASSUNG

Weibliche Mause wurden mit Dosen von 20 R oder 80 R am elften Tage der Schwangerschaft bestrahlt Die weiblichen Nachkommen wurden im Alter von 28 oder 56 Tagen getotet und die Anzahl von Oocyten und Follikeln wurde gezahlt Eine deutliche dosisabhangige Reduktion der Oocyten zwischen 20 % und 45 % im Vergleich zu den Kontrollen wurde festgestellt Die Reduktion war verhaltnismassig geringer bei den 56-Tage alten Mausen als bei den 28 Tage alten was auf einen Wiederholungsprozess deutet

RÉSUMÉ

Des souris femelles ont reçu au onzieme jour de leur gravidité des doses de rayons de Roentgen de 20 R ou de 80 R Les souriceaux femelles ont eté tués a l age de 28 ou de 56 jours et on a compte leurs oocytes et leurs follicules La reduction du nombre des oocytes est nettement en relation avec la dose et se situe entre 25 et 45 % par rapport aux sujets témoins Le fait que la reduction du nombre des oocytes est moins importante a l age de 56 jours qu a 28 jours fait penser qu il y a un processus de reparation

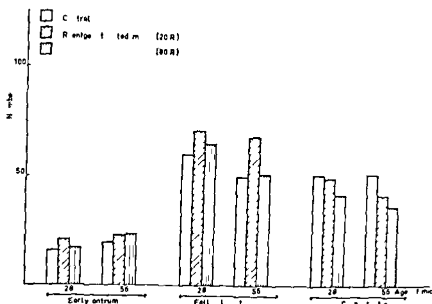


Fig 4 Average number of early antrum (cf text) and degenerated follicles

Results

The total number of cells and follicles per ovary among the irradiated mice was greatly diminished (Fig 1) compared with that of the control group It was also obvious when comparing irradiated with non irradiated mice that the relative decrease in cells was much more marked after 28 than after 56 days On the other hand, the reduction in the number of cells between day 28 and day 56 was greater in the control material than in the 20 R and 80 R groups (Fig 1)

The radiation induced reduction comprised all types of oocytes and follicles (Figs 2 and 3) but an opposite tendency was observed among the early antrum follicles and degenerating follicles (Fig 4)

The dose effect was revealed for some types of cells and follicles but not in all The difference between the dose effects seemed to be greater in the 28 day than in the 56 day old mice

Discussion

MINTZ (1959) irradiated female mice with 100 and 300 R at the same foetal age as we have used As the effect was studied 2 to 3 days after exposure the evaluation cannot be compared to our investigation In rats, however, a comparable experiment has been made by BEAUMONT (1962) She irradiated female rats at the foetal age of 10 and 12 days and counted the oocytes 25 days after birth There was a reduction of about 40 % following 50 R and 50 to 60 %

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EARLY EFFECTS OF LOCALIZED SINGLE DOSES OF IONIZING RADIATION ON HUMAN BONE MARROW

by

S STEFANI and A MONTI

Only sporadic observations are available on the effect of localized irradiation on the human bone marrow All have been carried out during and after repetitive therapeutic irradiations (GOSWITZ ANDREWS & KNISELEY 1963 HUTAFF & BELDING 1955 LEHAR et coll 1966 STEWART & DISCHE 1956 SYKES et coll 1960) To our knowledge there are no reports on the effect of single localized doses of ionizing irradiation on the differential cell count of human bone marrow in the immediate hours and days following exposure In order to supply this information the quantitative and qualitative changes in irradiated bone marrow of cancer patients were studied as a function of dose with constant time (two days after irradiation) and then as a function of time (4 hours 1 2 3 and 6 days) with constant dose (400 rad)

Material and Method Patients with bronchogenic carcinoma scheduled to receive radiotherapy were considered for this investigation Under study in our institution at present is the comparative therapeutic effect on bronchogenic carcinoma of daily treatments versus split weekly doses Patients receive either

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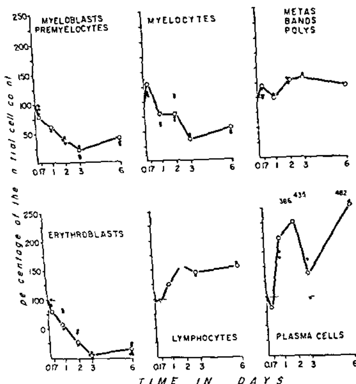


Fig 2 Marrow changes after 400 rad as a function of time expressed as a percentage of the initial count

chedule each patient had received a sternal tissue dose of 400 rad In addition the data from the 400 rad group of the first study (examination after two days) were also included with these results Included in this study were only patients who had no previous treatment specifically no antineoplastic drugs Furthermore, they must have shown no demonstrable clinical laboratory or radiologic evidence of bone metastasis In all cases studied the routine initial bone marrow aspiration revealed a bone marrow cellularity and an erythroid/myeloid cell ratio within normal limits

Results

The marrow changes in the three groups of five patients exposed to three doses of irradiation (200 400 800 rad) and examined after two days are illustrated in Fig 1 There is a drop in the myeloblast premyelocyte group which

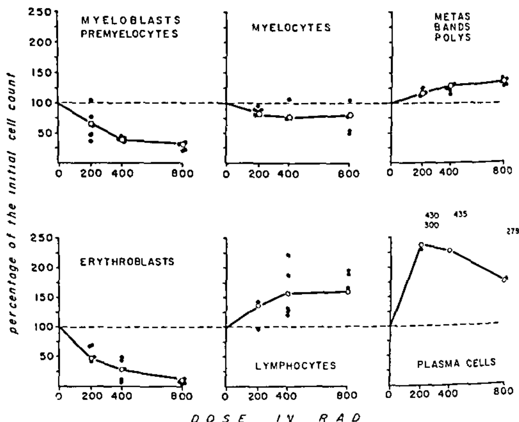


Fig 1 Relative bone marrow changes two days after localized irradiation as a function of dose Each point represents one patient open circles show the mean values

daily doses (four times a week) of 200 rad or single weekly doses of 400 or 800 rad, respectively, with a three field technique, resulting in a tissue dose of 200 400 or 800 rad ± 10 %, respectively to the center of the sternum The physical factors of the irradiation are 300 kV, 4 mm Cu HVL, 50 cm target skin distance and 48 R/min dose rate

The investigation was divided into two separate parts Part I was designed to investigate the effect of localized irradiation as a function of dose Fifteen patients were included in this study five from each dose group Two days after the initial exposure, permission was requested for a second bone marrow aspiration which the patients were told would be of experimental value only In the second part of the study, which was designed to evaluate the effect of localized irradiation as a function of time, 20 additional patients, all from the 400 rad weekly split dose group, were included Again, two aspirations were performed on each, one routinely before irradiation and another granted either ½ hours, 1, 3 or 6 days after the first set of treatments At this point in the radiotherapy

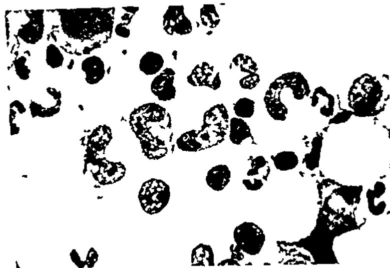


Fig 4 Giant band observed in a smear obtained 3 days after exposure to 400 rad Complete absence of erythroblasts and presence of several lymphocytes ×785

bone marrow regeneration because we also found in the 6 day smears basophilic and orthochromatic normoblasts which were not found in any of the smears obtained 2 and 3 days after irradiation After an apparent slight initial drop at 4 hours both the lymphocytes and plasma cells later rose and remained above the initial count throughout the entire investigation (6 days) As in the first experiment the variations were wider in the plasma cells than in the lymphocytes

No morphologic abnormalities were seen in the 4 hour smears They were however seen in all smears taken 1 2 and 3 days after irradiation For the erythroblasts these were characterized by an increase in cell size disruption of the nuclear chromatin pattern presence of nuclear fragments in the cytoplasm and by double nuclei sometimes connected by small chromatin bridges (Fig 3) The changes in the myeloid cells were characterized by marked increase in cellular size in some of the metamyelocytes and bands (Fig 4) by the presence of toxic granulations in the cytoplasm and of finely dispersed chromatin in the nucleus and by hypersegmentation of the polymorphonuclear cells At 6 days abnormalities were not noted in the erythroblasts but were still present in the myeloid series No morphologic alterations could be detected at any time in the lymphocytes or plasma cells although unrecognizably distorted and damaged cells were noted in all irradiated smears It is interesting to note that two days after irradiation there appeared to be no connection between the number of

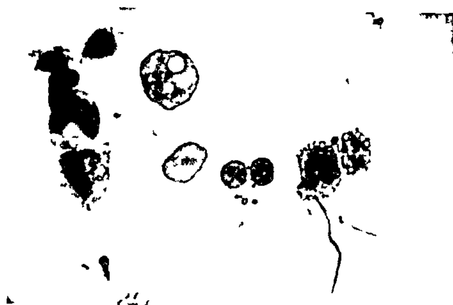


Fig. 3. Orthochromatic normoblasts have increased cell size, double nucleus connected by chromatin bridges and nuclear fragments in the cytoplasm. Bone marrow obtained 2 days after exposure to 400 rad. ×1025

appears to be more marked at the higher dosages. There is also a drop in the mean relative percentage of the myelocytes, and a relative increase in the more mature myeloid cells, the latter again appearing more marked at the two higher dosages. The most marked changes, however, were observed in the erythroblasts. The mean relative drop in these cells grew larger with the roentgen dose, the mean reaching a low point of 10.8% of the initial count after 800 rad. The relative percentage of lymphocytes rose strikingly, the mean being 137.3 for 200 rad, 157.1 for 400 rad, and 159.8 for 800 rad. The relative percentages of plasma cells followed a pattern similar to that of the lymphocytes, however they showed wider variations, due probably to the small initial count.

The changes produced by 400 rad as a function of time are recorded in Fig. 2. There was a relative drop in the myeloblast-premyelocyte group which was already evident at 4 hours, reached its lowest point at 3 days, and manifested some signs of recovery at 6 days. After an initial relative increase at 4 hours, the myelocytes followed a similar pattern but with a delay of about one day. The relative percentage of the maturing myeloid cells increased in 22 out of 25 patients and showed no clear dependency on the time of examination. The erythroblasts revealed the sharpest relative drop of any cell component. The drop began at 4 hours and, as in the myeloid precursors, reached a very low point at three days (mean about 4% of the initial count) and showed some signs of recovery at 6 days. We interpreted this later increase as a sign of initial

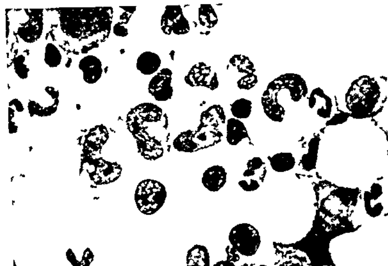


Fig 4 Giant bands observed in a smear obtained 3 days after exposure to 400 rad Complete absence of erythroblasts and presence of several lymphocytes × 85

bone marrow regeneration because we also found in the 6 day smears basophilic and orthochromatic normoblasts which were not found in any of the smears obtained 2 and 3 days after irradiation After an apparent slight initial drop at 4 hours both the lymphocytes and plasma cells later rose and remained above the initial count throughout the entire investigation (6 days) As in the first experiment the variations were wider in the plasma cells than in the lymphocytes

No morphologic abnormalities were seen in the 4 hour smears They were however seen in all smears taken 1 2 and 3 days after irradiation For the erythroblasts these were characterized by an increase in cell size disruption of the nuclear chromatin pattern presence of nuclear fragments in the cytoplasm and by double nuclei sometimes connected by small chromatin bridges (Fig 3) The changes in the myeloid cells were characterized by marked increase in cellular size in some of the metamyelocytes and bands (Fig 4) by the presence of toxic granulations in the cytoplasm and of finely dispersed chromatin in the nucleus and by hypersegmentation of the polymorphonuclear cells At 6 days abnormalities were not noted in the erythroblasts but were still present in the myeloid series No morphologic alterations could be detected at any time in the lymphocytes or plasma cells although unrecognizably distorted and damaged cells were noted in all irradiated smears It is interesting to note that two days after irradiation there appeared to be no connection between the number of

cells with morphologic abnormalities and the dose of irradiation received by the bone marrow.

Discussion

No attempt was made to determine the absolute bone marrow count, due to the well known unreliability of such determinations on aspirated material. For this reason, although it is quite probable that all the observed percentage changes represent cell destruction, one cannot exclude the possibility of cell migration to or away from the irradiated areas. The erythroblasts and, to a lesser extent, the young cells of the myeloid series showed the most marked relative drop in the early days following irradiation. This is in the keeping with the well established radiosensitivity of these elements (DENSTAD 1943). Moreover, the mean percentage changes in the erythroblasts seemed to be related to the dose of irradiation and to the time after irradiation, at least for the doses and times used. One can wonder whether repetitive bone marrow aspirations in the hours and immediate days after an accidental localized radiation would be of some use in approximating the degree of exposure. This, of course, still remains to be demonstrated.

The observation of a relative increase in the percentage of lymphocytes was quite surprising, considering the well established high radiosensitivity of the blood lymphocytes. However, this observation in humans is not unique since it has been reported after repetitive localized irradiations (GOSWITZ et coll. 1963, HUTAFF & BELDING 1955, LEHAR et coll. 1966, STEWART & DISCHE 1956). Furthermore, similar findings have been described after whole body irradiation in humans and experimental animals. An increased percentage of lymphocytes was found in the bone marrow obtained after 3 to 5 weeks in humans exposed to the irradiation of the atomic bomb (OUGHTERSON & WARREN 1956). In individual cases, there were levels as high as 80 % and most of the cells appeared to be normal small lymphocytes. An absolute increase in the marrow lymphocyte count in mice 10 days after 400 R whole body irradiation has been observed by BRECHER et coll. (1948). Similarly, HARRIS (1956) found an increase in the absolute number of bone marrow lymphocytes in guinea pigs exposed 10 days previously to sublethal doses of whole body irradiation. From sequence of changes in the other bone marrow cell components, he found support for the hypothesis of MAXIMOW, who first considered the lymphocyte to be a stem cell.

The observed relative increase of lymphocytes in our experiments could be the result of repopulation of the irradiated sternal area with normal lymphocytes migrating from non irradiated sites. According to our observations this migration, if present, occurs promptly, being fully manifest already one day after irradiation. Another possibility is that the bone marrow lymphocytes are relatively more

radioresistant than other bone marrow cells and also more radioresistant than the blood lymphocytes. This hypothesis could be related to the findings of STEWART & DISCHE (1956) who noted a relative lymphocytosis in the bone marrow accompanied by marked lymphopenia in the blood of six patients with ankylosing spondylitis treated 1 to 189 days previously with localized irradiation. The suggested relative radioresistance of the marrow lymphocytes would give some support to the hypothesis, based on in vitro work, that there are two lymphocytic cell populations, one radiosensitive and one radioresistant (SCHREK & STEFANI 1964). Also this observation would have some implication in organ transplantation. Whole body irradiation has been used for suppressing the immunologic defenses of organ recipients, i.e. to destroy the lymphocytes now well recognized to be the immunologic competent cell. It is obvious that if this cell is indeed more radioresistant than other bone marrow components, an attempt at immunosuppression by use of radiation would be comparatively ineffective.

Acknowledgement

We wish to thank Dr. Harold Schoolman for his invaluable suggestions, Dr. William Neville for his kind collaboration and Mrs. Zielonka and Mr. Kirsh for their technical assistance.

SUMMARY

The effect of localized single doses of ionizing irradiation on normal bone marrow of patients with bronchogenic carcinoma was studied. The studies were made as a function of dose and of time. Quantitative modifications consisted of a marked percentage decrease in the erythroblasts and a less marked percentage decrease in the precursors of the granulocytic series accompanied by a shift to the right. A noticeable relative increase in lymphocytes and plasma cells starting one day post irradiation was observed.

ZUSAMMENFASSUNG

Der Effekt einer lokalisierten Einzel-Dosis ionisierender Strahlung auf das normale Knochenmark von Patienten mit bronchogenen Carcinomen wurde studiert. Die Untersuchungen wurden als Funktion der Dosis und der Zeit ausgeführt. Quantitative Veränderungen bestehen aus einem kräftigen prozentuellen Abfall der Erythroblasten und einem weniger ausgeprägten prozentuellen Abfall der Vorstufen der granulozytären Reihe, verbunden mit einer Rechts-Verschiebung. Ein bemerkenswerter relativer Anstieg der Lymphozyten und Plasmazellen, der einen Tag nach der Bestrahlung einsetzte, wurde beobachtet.

RÉSUMÉ

Les auteurs ont étudié l'effet des doses uniques localisées d'irradiation ionisante sur la moelle osseuse normale de malades atteints de cancer bronchique. Ces études ont été faites en fonction de la dose et du temps. Les modifications quantitatives ont consisté en une dimi

nution importante du pourcentage des erythroblastes et en une diminution moins importante des precurseurs de la serie granulocytaire accompagnee par une deviation vers la droite Les auteurs ont observe une augmentation relative des lymphocytes et des cellules plasmocytaires commençant un jour apres l irradiation

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FROM THE MEDICAL RESEARCH COUNCIL'S RADIOBIOLOGY UNIT, HARWELL DIDCOT
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DEPIGMENTATION OF HAIR IN TWO STRAINS OF MICE AFTER SINGLE AND FRACTIONATED DOSES OF ROENTGEN RAYS

by

E V HULSE and L G MIZON

Depigmentation of hair has usually been studied as a response occurring a few weeks after irradiation and experiments have entailed plucking hair to synchronise hair cycles and often to measure the radiation response (CHASE 1948 1949 POTTEN 1968 POTTEN & HOWARD 1969) The present experiments using roentgen rays were intended to supplement observations on the effects of beta irradiation of mouse skin (HULSE 1967 HULSE & MOLE 1969 HULSE DEMPSEY & BATCHELOR unpublished) and were designed to follow the process of depigmentation over several months with as little interference as possible Consequently hairs were not plucked out at any stage and apart from the irradiations hair growth was allowed to proceed naturally Part body irradiation was used to avoid any general effects

Methods

Mice Female CBA/H and C57Bl/H mice aged 6 to 9 months were randomized and kept 4 to a cage The numbers of mice used at each dose level and type of fractionation are given *in the* Table

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Table

Number of mice exposed to single doses or two equal fractions of various doses of roentgen rays and the time between the fractions

Strain	Total dose (rad)	Number of fractions	Time between fractions (hours)	Number of mice irradiated	Number of mice alive at 48 weeks
CBA	500	1	—	16	14
	500	2	6	16	16
	500	2	24	16	15
	250	1	—	16	15
C57Bl	750	1	—	4	3
	500	1	—	8	8
	500	2	6	8	8
	500	2	24	8	7
	400	1	—	4	4
	250	1	—	8	8

Irradiations. A 2 cm transverse zone across the middle of the torso was irradiated. The mice were exposed in separate perspex tubes, 2.5 cm internal diameter, with perforations at one end to allow a plentiful supply of fresh air. The closures of the tube, at the tail end, were adjusted so that the mouse was kept in one position during the irradiation. The mice were irradiated dorso-ventrally, four at a time, and, except for the 2 cm gap over the mid torso, were shielded by lead sheets 6 mm thick. The irradiation factors were 250 kV, 135 rad/min with a HVL of 1.2 mm Cu.

Scoring the degree of depigmentation. The mice were examined weekly for 48 weeks and the degree of depigmentation of the dorsal aspect of the irradiated zone was judged from the general overall naked eye appearances. The system of scoring was originally evolved to study effects of beta irradiation (HULSE et coll., unpublished). Normal hair without any depigmentation scored zero. An irradiated zone in which the hair was completely white without any evidence of natural colour scored 8. Intermediate stages received appropriate scores in the range 1 to 7, e.g., when careful examination revealed a few depigmented hairs in the irradiated zone a score of 1 was given; when the colour of the irradiated zone appeared about half way between normal and complete depigmentation the score was 4; and when only a few scattered pigmented hairs prevented the zone being judged as completely white a score of 7 was

given Weekly means and standard errors were calculated for each irradiation group

In order to ensure that the scoring was consistent animals which had received the lower doses were always examined first A few scattered white hairs are normally present in C57Bl/H mice and these were disregarded the unirradiated skin providing a standard for judging their incidence Neither strain spontaneously develops depigmented hair during the age range covered by this experiment

Results

CBA mice (Fig 1) After a single dose of 500 rad depigmentation was first noticed at 7 weeks after irradiation It progressed steadily to 23 weeks and then remained more or less unchanged until 36 weeks when a further slight increase in depigmentation started A single dose of 250 rad did not produce any discernible depigmentation

When 500 rad was given in two fractions the onset of visible depigmentation was postponed and the degree of depigmentation was consistently less the 24 hour interval between doses reducing the effect and postponing the onset more than the 6 hour interval From 25 to 27 weeks onwards the degree of depigmentation remained steady until a second minor increase occurred at 40 weeks after the 6 hour interval and at 47 weeks after the 24 hour interval

C57Bl mice (Fig 2) After single doses of 400 to 750 rad the degree of depigmentation its rate of development and its time of onset were dose dependent Again no depigmentation occurred after 250 rad Dividing 500 rad into two fractions delayed the onset and reduced the degree of depigmentation below that which occurred after a single dose of 400 rad A 24 hour interval between fractions again reduced the effect more than a 6 hour interval The degree of depigmentation remained static from 25 to 30 weeks onwards in all groups except those given a single dose of 400 rad which showed a slight but non significant increase from 43 to 46 weeks

Discussion

The present investigation differs from those of previous workers in the following ways (1) measurements of depigmentation were rapid and did not require any special skill or apparatus (2) the mice were irradiated when several months older than usual e g 4 to 8 months older than POTTEN's (1968) and (3) measurements for the dose response curve (Fig 3) were taken almost one year after irradiation rather than at a few days or weeks

The response of the hair follicle to radiation varies according to the stage of the hair cycle at the time of exposure Depigmentation is greater when resting

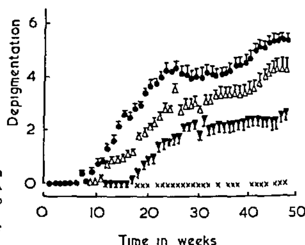


Fig. 1. Average weekly depigmentation scores with standard errors for CBA mice. Single doses of 500 rad ●; two doses of 250 rad with a 6 hour interval △ and with a 24 hour interval ▼; single dose of 250 rad ×.

(telogen) hair is exposed and delay in hair growth is greater when growing (anagen) hair is irradiated (CHASE 1949). However, variations in sensitivity during the hair cycle are not important in the present investigation. The hair of mice aged 6 to 9 months is in the resting phase most of the time and the likelihood of irradiating unplucked hair follicles during anagen is very slight; indeed, CHASE (1949) found that depigmentation following the irradiation of unplucked follicles was the same as that following the irradiation of follicles known to be in telogen. Post irradiation delay in hair growth would be minimal in resting hairs, and our observations extended over many months. Thus, any temporary delay in hair growth did not interfere with the assessment of final depigmentation, which in most cases was stationary over several weeks.

The present observations (Figs 1 and 2) confirm that hair depigmented by ionizing radiation does not regain its colour, even after many months (DANEEL & LUBNOW 1936, CHASE 1949). The second increase in depigmentation seen in CBA mice at 40 weeks after irradiation (Fig. 1) corresponds to that seen in F_1 (C57L × A/He) mice about one year after exposure to gamma rays from an atom bomb (UPTON et coll. 1960). The absence of a second increase in depigmentation in C57Bl mice (Fig. 2) is in agreement with CHASE's (1949) observations.

Sensitivity of C57Bl and CBA mice. Apart from the difference in the delayed increase in depigmentation, the response of the two strains was very similar, e.g. at 40 weeks the scores for 500 rad given as a single dose or as two fractions with a 6 hour or a 24 hour interval were about 5, 3 and 2 respectively in both strains (Figs 1 and 2). This confirms that strain differences are slight after a single dose (POTTEN 1969) and also shows that the same applies for depigmentation after fractionation.

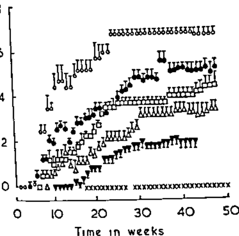


Fig 2 Average weekly depigmentation scores with standard errors for C57Bl mice Single dose of 750 rad ○ of 500 rad ● of 400 rad □ two doses of 250 rad with a 6 hour interval △ and with a 24 hour interval ▼ single dose of 250 rad × (Data on two doses of 250 rad with a 24 hour interval cease at 43 weeks because three of the remaining seven mice developed generalised alopecia which is not uncommon in aged C57Bl/H mice The degree of depigmentation did not alter in the remaining mice of the group)

Threshold dose Irradiation with 250 rad was below the threshold for hair epigmentation in CBA and C57Bl mice (Figs 1 and 2) and similar threshold alues have been obtained previously in C57Bl and in F_1 (C57L × A/He) nice (Chase 1949 Upton et coll 1960) Each hair follicle is supplied with a umber of melanocytes and the finding of a threshold dose for the depigmenta ion of the hair does not imply that no melanocytes whatsoever are permanently ffected by lower doses Nevertheless there were only minimal changes in counts of hair follicle melanocytes after 250 rad of 300 kV roentgen rays (Potten 1968)

Survival curves In the present investigation depigmentation was judged by assessing the colour of the irradiated zone and no attempt was made to count depigmented hairs or changes in the number of melanocytes Nevertheless the data can be used to obtain a dose response curve by subtracting the final score of a group of mice from the maximum possible (i e 8) and expressing this as a fraction of 8 The resulting type C survival curve for C57Bl mice is given in Fig 3 The D_{37} is 260 rad and the extrapolation number 2 5 These values are similar to those for resting hairs obtained by more refined techniques measurements of the mean number of melanocytes per follicle gave $D_{37} = 180$ —220 rad (Potten 1968) and counts of the number of follicles with one or more melanocytes gave $D_{37} = 346$—656 rad and $n = 2$ (Potten 1968 Potten & Howard 1969) Chase (1949) did not construct survival curves but his data on resting hairs can be dealt with in the same way as our data (Fig 3) For 200 kV roentgen rays $D_3 = 300$ R and $n = 2$ and for 100 kV roentgen rays $D_3 = 280$ R and $n = 2 5$

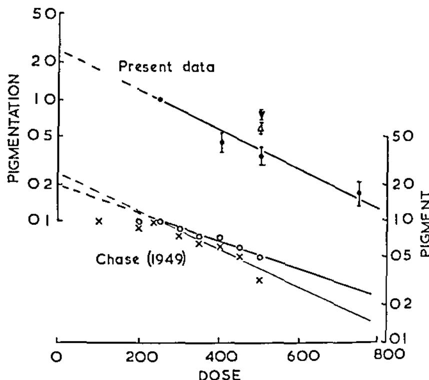


Fig. 3. *Upper curve and left hand ordinate scale.* Survival curve for hair colour remaining in C57Bl mice at the end of present experiment (doses in rad). Single doses ●; two doses of 250 rad with a 6 hour interval △ and with a 24 hour interval ▼.

Lower curves and right hand ordinate scale. Survival curves for hair colour remaining in CHASE's (1949) C57Bl mice irradiated during the resting phase of hair growth (appropriate data calculated from Tables 1 and 2 of CHASE (1949) with doses in R). ○ denotes 200 kV roentgen and × 100 kV roentgen radiation.

Fractionation. Repeated exposures to subthreshold doses produced depigmentation (DANEEL & LUBNOW 1936, CHASE & RAUCH 1950). In the present experiments two subthreshold exposures, to either strain, produced depigmentation. In no case was the effect as great as it would have been if the two doses had been given consecutively without a radiation-free interval. However, the amount of depigmentation was greater with the shorter interval between doses (Figs. 1 and 2).

As read from the graph (Fig. 3) the single dose (D_1) which would have produced the same effect as 500 rad given as two equal fractions (D_2) is 390 rad for a 6 hour interval between fractions and 360 rad for a 24 hour interval. Thus $(D_2 - D_1)$ 6 hours = 110 rad and $(D_2 - D_1)$ 24 hours = 140 rad. CHASE (1948) found that $(D_2 - D_1)$ 3 days = 200 R.

Acknowledgements

We are very grat ful to Mr and Mrs M J Corp for the irradiations

SUMMARY

Depigmentation of hair after part body irra liation of mice was judged visually at weekly intervals The onset rate of increase and final degree of depigmentation at 25 to 40 weeks were do e dependent The thre hold was above 250 rad Di iding a dose into two equal fractions decreased the degree of depigmentation a 24 hour interval was more effective than one of 6 hours The observations allowed values for melanocyte survival to be deduced (D_{37} 260 rad $n = 2\,5$) For fractionated doses D_2—D_1 was 110 to 140 rad.

ZUSAMMENFASSUNG

Di Depigmentierung der Haare nach Teilkorperbestrahlung von Mausen wurde visuell in wo h ri chen Intervallen festgestellt Der Beginn die Geschwindigkeit des Anstiegs und d r s hliessliche Grad der Depigmentierung zwischen 25 und 40 Wochen sind Dosis-abhangig Die Schwelle liegt uber 250 rad Teilt man die Dosis in zv ei gleiche Fraktionen sinkt der Grad der Depigmentierung ein 24 Stunden Intervall ist effektiver als ein 6 Stunden Intervall Die Beobachtungen erlauben Überlebenswerte fur die Melanozyten herzuleiten (D_{37} = 260 rad n = 2 5) Fur fraktionierte Dosen war D_2—D_1 110 bis 140 rad

RÉSUMÉ

Les auteurs ont apprecie visuellement à intervalles hebdomadaires sur deux souches de souris la dépigmentation des poils apres irradiat on d une partie du corps Le debut la vites e d accroissement et le degre final de depigmentation entre 25 et 40 semaines dependent de la dose Le seuil est au dessus de 250 rad La division de la dose en deux fractions egales diminue l degre de depigmentation un interval e de 24 heures a plus d effet qu un intervalle de 6 heures Les observations ont permis de deduire des valeurs de survie des melanocytes (D_{37} 260 rad $n = 2\,5$) Pour les doses fractionnees D_2—D_1 est compris entre 110 et 140 rad

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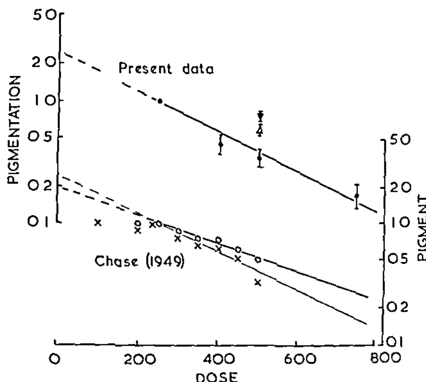


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As read from the graph (Fig. 3) the single dose (D_1) which would have produced the same effect as 500 rad given as two equal fractions (D) is 390 rad for a 6 hour interval between fractions and 360 rad for a 24 hour interval. Thus $(D - D_1)$ 6 hours $=$ 110 rad and $(D - D_1)$ 24 hours $=$ 140 rad. CHASE (1948) found that $(D - D_1)$ 3 days $\simeq$ 200 R.

SERVICE DE CURIETHERAPIE (PROF AGR B PIERQUIN) INSTITUT GUSTAVE ROUSSY
VILLEJUIF FRANCE

CARCINOMES EPIDERMOIDES DE LA LANGUE MOBILE ET DU PLANCHER BUCCAL

Etude de 245 cas traites a l Institut Gustave Roussy

par

B PIERQUIN D CHASSAGNE Y CACHIN F BAILLET et F FOURNELLE LE BUIS

Notre etude porte sur 245 cas de carcinomes epidermoides de la langue mobile et du plancher buccal (T1, T2 et T3) traites entre le debut de 1960 et la fin de 1965 Nous disposons donc d un recul d observation minimale superieur a 3 ans

Le but principal de ce travail est d apprecier les resultats de l endocurietherapie par iridium 192 avec nos nouvelles techniques de preparation non radio active en les comparant aux resultats de la technique classique d endocurietherapie par radium (BAILLET 1968)

Donnees cliniques Nous avons regroupe tous les cas de carcinomes epidermoides interessant les muqueuses des parties molles se situant a l interieur de l arc mandibulaire nous associons donc les lesions de la langue mobile et celles du plancher buccal en excluant les localisations tumorales debordant dans la base de langue sur plus d un centimetre d un part envahissant directement la muqueuse gingivale d autre part Pratiquement cette delimitation revient a etudier tous les cancers interessant exclusivement les parties molles comprises dans l arc mandibulaire ce qui revient a eliminer les tumeurs marginales d une

Soumis à la Redaction le 28 Juillet 1969

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Tableau 1

Repartition des cas (au total 245) selon le systeme TNM

	N0	N1	N2	N3	Total
T1	18	2			20
T2	68	33	7	11	119
(T2a)	(30)	(14)	(5)	(?)	(51)
(T2b)	(38)	(19)	(2)	(9)	(68)
T3	48	31	8	19	106
(T3)	(27)	(19)	(3)	(7)	(56)
(T3b)	(21)	(12)	(5)	(12)	(50)
Total	134	66	15	30	245

des tumeurs primitives ainsi que leurs sous-groupes (T2 et T3) se repartissent en nombres a peu pres equivalents d autre part que les adenopathies clinique ment apparentes avant le traitement sont dans les memes groupes et sous groupes de l ordre de 50 %

Traitement

Conduite generale pour les malades des groupes T1 T2 et T3a Le traitement se compose (1) d une endocurietherapie de la tumeur primitive (radium ou iridium 192) dans un premier temps (2) en cas d adenopathie cliniquement palpable d une chirurgie avec curage ganglionnaire sous-maxillaire bilateral et jugulo-carotidien uni ou bilateral en fonction de l extension metastatique uni ou bilaterale au niveau du ganglion de Kutner (3) en cas d adenopathie histologiquement confirmee (N+) d une teleradiotherapie des aires lymphati ques cervicales droite et gauche (3 500 rad pour les cas traites par tele roentgentherapie sous 200 kV 5 000 a 6 000 rad pour les cas traites par telecobalt)

Si aucune adenopathie n est cliniquement perceptible le traitement se limite a l endocurietherapie de la tumeur primitive une surveillance reguliere des aires lymphatiques s exerce ensuite tous les deux ou *trois mois* si une adeno-pathie secondaire apparait le curage chirurgical suivi de l eventuelle teleradio therapie sont alors aussitot appliques

Conduite generale pour les malades du groupe T3b Ce protocole thera peutique n est modifie qu en fonction de la *tumeur* primitive du fait de l importance de l exten ion tumorale une teleradiotherapie par telecobalt precede l endocurietherapie (4 500 rad) l endocurietherapie ne s applique qu au reliquat tumoral (4 000 rad)

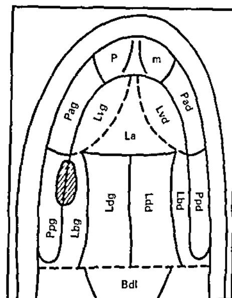


Fig 1 Schéma topographique de la langue et du plancher Exemple d un carcinome epidermoide pelvi lingual (Ppg et Lbg T2a)
Pm — plancher médian
Pag — Pad — plancher anterieur gauche ou droit
Ppg — Ppd — plancher posterieur gauche ou droit
La — langue anterieure
Lvg — Lvd — langue ventrale gauche ou droite
Lbg — Lbd — bord de langue gauche ou droit
Ldg — Ldd — langue dorsale gauche ou droite
Bdl — base de langue

part, les tres grosses tumeurs debordant sur les structures avoisinantes d autre part (T4)

Tous nos cas sont des lesions neuves, sans traitement local anterieur, sans autre localisation tumorale simultanement associee Sur ces 245 cas, nous denombrons 230 hommes et 15 femmes La moyenne d'age est de 57 ans Dans la tres grande majorite des cas, il s agit de sujet tares, ethyliques et disposant d une mauvaise dentition

Nous avons classe l extension locale et ganglionnaire selon le systeme TNM, a savoir, *pour la tumeur primitive*

T1 — tumeur de moins de 1 cm, sans infiltration sous muqueuse palpable,

T2 — tumeur entre 1 et 3 cm, avec infiltration sous muqueuse inferieure a 1 cm,

T3 — tumeur entre 3 et 5 cm, avec une infiltration inferieure a 3 cm

et *pour les adenopathies*

N0 — pas d adenopathie palpable,

N1 — adenopathie(s) mobile(s) et homolaterale(s),

N2 — adenopathie(s) mobile(s) controlaterale(s) ou bilaterale(s),

N3 — adenopathie(s) fixee(s)

Nous avons adjoint, pour la tumeur primitive, une etude topographique basee sur un quadrillage delimitant 13 secteurs (voir figure 1)

Cette etude topographique jointe a celle de l extension nous a permis de definir deux sous groupes pour les T2 et les T3 T2a, T2b, T3a, et T3b

La repartition de nos 245 cas selon le systeme TNM est donnee sur le Tableau 1 On constate d une part que les deux groupes les plus importants

Tableau 3

Distribution de la survie des cas en fonction de l'extension locale et ganglionnaire (normalisee a 3 ans d observation)

	N0	N1	N2	N3	Total	
T1	14/18	2/2			16/20	(80 %)
T2	31/68	12/33	4/7	3/11	50/119	(42 %)
T3	22/48	14/31	3/8	7/19	46/106	(43 %)
Total	67/134 (50 %)	28/66 (42 %)	7/15 (46 %)	10/30 (33 %)	112/245	(46 %)

avec tubes plastiques et fils radio-actifs (fils d iridium 192 de 0 3 mm de diametre) Ces techniques ont ete decrites dans d autres travaux (PIERQUIN 1964 1969)

Grosso modo la technique par gouttieres vectrices est reservee aux tumeurs T1 T2 et T3a cependant que la technique par tubes plastiques est reservee aux tumeurs T3b La dose tumorale calculee par la technique sur points (PIERQUIN et coll 1962) est de 7 000 rad dans les cas traites par la seule endocurietherapie de 4 000 rad dans les cas traites par association teleradiotherapie endocurietherapie cette dose tumorale est calculee sur une isodose de reference dont la valeur se situe entre 80 et 90 % de celle de la dose de base Le debit quotidien est de l ordre de 1 000 a 1 500 rad ce qui donne pour une dose totale de 7 000 rad une duree d application de 5 a 7 jours

La repartition des cas traites soit par radium soit par iridium 192 en fonction de l extension tumorale est presentee sur le Tableau 2

On constate une repartition tres differente de ces deux modalites d endocurietherapie en fonction de l extension tumorale Grosso-modo les indications de l endocurietherapie par radium ont interesse des lesions moins etendues que celles traitees par iridium 192

Resultats

La survie de nos 245 cas est normalisee a 3 ans d observation Sa distribution en fonction de l extension locale et ganglionnaire apparait sur le Tableau 3

Ces taux de survie font apparaitre trois faits (1) le taux de survie des T1 est tres eleve (80 %) la plupart des T1 ne presentent pas d adenopathie au moment du traitement (2) les taux de survie des T2 et des T3 sont identiques entre 1 et 5 cm les carcinomes epidermoides de la langue mobile et du plancher buccal ont un pronostic identique (3) la presence ou l absence d adenopathie clinique apparente au moment du traitement ne modifie pas fondamentalement

Tableau 2

Répartition des cas traités soit par radium, soit par iridium 192 en fonction de l'extension tumorale

	Radium	Iridium	Pas d'endocurie	Total
T1	17	2	1	20
T2	90	27	2	119
(T2a)	(44)	(7)	(0)	(51)
(T2b)	(46)	(20)	(2)	(68)
T3	32	66	8	106
(T3a)	(22)	(32)	(2)	(56)
(T3b)	(10)	(34)	(6)	(50)
Total	139	95	11	245

Modalités de l'endocuriethérapie. L'ensemble de nos malades a été traité selon deux modalités d'endocuriethérapie : (1) utilisant les aiguilles de radium, et (2) utilisant des fils d'iridium 192.

1. Il s'agit d'une technique classique utilisant des aiguilles d'assez faibles longeurs radio-active (entre 0,6 et 1,7 cm) avec une activité linéaire de l'ordre de 1,33 mCi/cm. L'implantation s'effectue sous anesthésie générale. La disposition du matériel radifère reste habituellement parallèle à la surface muqueuse en cas de lésion non infiltrante, perpendiculaire à cette surface en un ou deux plans en cas de lésion infiltrante. Pour des lésions centrées autour du sillon pelvi-lingual, l'endocuriethérapie s'effectue le plus souvent en deux temps (un temps lingual et un temps pelvien) successifs, dans tous les autres cas, en un seul temps. Pour les lésions du plancher buccal, un appareillage de contention en matière plastique avec appui sur la mandibule et fixation par suture cutanée maintient le dispositif radifère en place (technique de H. Kritter, VIGNIER 1961).

La dosimétrie reste basée sur des règles empiriques, basées sur l'expérience clinique et calculée en milligrammes-heures. Un certain nombre de contrôles ont permis de constater d'assez grandes variations dans la dose tumorale d'un cas à un autre : grosso modo, cette dose est très élevée dans les petits volumes cibles (T1 et T2a), de l'ordre de 10 000 rad, relativement faible et surtout inhomogène dans les volumes cibles de moyenne importance (T2b et T3), de l'ordre de 5 000 à 6 000 rad. La durée d'application est, le plus souvent, de six jours.

2. Avec l'endocuriethérapie par fils d'iridium 192 il s'agit de deux modalités techniques avec préparation non radio-active, l'une avec gouttières vectrices et épingles radio-actives (fils d'iridium 192 de 0,5 mm de diamètre), l'autre

Tableau 5

Comparaison entre les resultats des deux modalites 1 endocurietherapie par la frequence des recidives (ou des non sterilisations) de la tumeur primitive

	Radium	Ir d um
T1	3/17 (17)	0/?
T2	30 90 (33)	1/27 (4)
(T2a)	(13/44)	(0/7)
(T2b)	(17/46)	(1/20)
T3	14/3? (43)	11/6? (16)
(T3)	(9/22)	(2/32)
(T3b)	(/10)	(7/34)

Il existe donc une difference moderee en faveur de l iridium 192 ou le taux de survie pour les T2 et les T3 atteint 50 % alors qu il ne depasse pas 40 % avec le radium Nous discuterons plus loin de la signification de cette difference

Une comparaison plus precise entre les resultats de ces deux modalites d endocurietherapie peut etre etablie en etudiant la frequence des recidives (ou des non sterilisations) de la tumeur primitive observee a 3 ans Le Tableau 5 chiffre ces taux de recidives

Les differences dans la frequence des recidives entre radium et iridium 192 sont tres importantes pour les T2 les recidives avec l iridium 192 sont huit fois moins frequentes pour les T3 elles sont trois fois moins frequentes Cette difference est encore plus frappante si l on compare les malades qui n ont subi que la seule endocurietherapie c est a dire les malades des groupes T1 T2 et T3a (lesions de moins de 4 cm de longueur et de 2 cm d infiltration) on observe les taux suivants

	Rad um	Ir dium
T1 T2 T3a	42/129	3/61
Taux bruts	33 %	5 %
Taux ponderes	35 %	3 %

Une conclusion s impose pour les cancers de la langue mobile et du plancher buccal les recidives sont dix fois moins frequentes au niveau de lesions de moins de 4 cm lorsque l on utilise l iridium 192 en preparation non radio-active selon une dosimetrie bien controlee au lieu du radium selon une dosimetrie empirique

Resultats en fonction des adenopathies La survie en fonction des N0 N1 N2 et N3 n accuse pas de differences tres importantes Mais ces resultats appa

Tableau 4

Les taux de survie à 3 ans en fonction du traitement de la tumeur primitive

	Radium	Iridium	Pas d'endocurie	Total
T1	14/17	1/2	1/1	16/20
T2	37/90	13/27	0/2	50/119
(T2a)	(21/44)	(4/7)		(25/51)
(T2b)	(16/46)	(9/20)	(0/2)	(25/68)
T3	14/32	32/66	0/8	46/106
(T3a)	(11/22)	(18/32)	(0/2)	(29/56)
(T3b)	(3/10)	(14/34)	(0/6)	(17/50)
Total	65/139	46/95	1/11	112/245

le pronostic (si l'on met à part les T1, le pronostic des N0 est identique à celui des N1 et des N2, pour T2 et les T3).

Il convient d'étudier plus en détail ces résultats globaux, en fonction du traitement de la tumeur primitive d'un part, et en fonction des adénopathies d'autre part.

Résultats en fonction du traitement de la tumeur primitive. Les taux de survie à 3 ans, en fonction du traitement de la tumeur primitive apparaissent sur le Tableau 4.

Globalement, ces taux de survie font apparaître des résultats identiques entre radium et iridium. Mais la répartition des deux techniques entre les T1, les T2 et les T3 est trop inégale pour tirer une conclusion valable à partir de ces totaux. Ceci est particulièrement évident pour les T1 qui ont été presque tous traités par radium et dont les conditions cliniques et pronostiques apparaissent très différentes (adénopathies exceptionnelles et taux de survie très élevé).

Un essai de comparaison entre la survie des cas traités par radium et ceux traités par iridium 192 ne peut être tenté que pour les T2 et les T3 dont les conditions cliniques et pronostiques sont très comparables. En reprenant les résultats du Tableau 4 pour les T2 et les T3, on obtient les taux suivants:

	Radium	Iridium
Taux bruts	42 %	48 %
Taux pondérés	40 %	50 %

Les taux pondérés sont obtenus en supposant que tous les malades (T2a, T2b, T3a et T3b) ont été traités soit par radium, soit par iridium 192.

Tableau 5

Comparaison entre les resultats des deux modalites d endocurietherapie par la frequ nce des recidives (ou des non sterilisations) de la tumeur primitive

	Radium	Irid m
T1	3/17 (17)	0/2
T2	30/90 (33)	1/27 (4)
(T2a)	(13/44)	(0/7)
(T2b)	(17/46)	(1/20)
T3	14/32 (43)	11/66 (16)
(T3a)	(9/22)	(2/32)
(T3b)	(5/10)	(7/34)

Il existe donc une difference moderee en faveur de l iridium 192 ou le taux de survie pour les T2 et les T3 atteint 50 % alors qu il ne depasse pas 40 % avec le radium Nous discuterons plus loin de la signification de cette difference

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Tableau 4

Les taux de survie à 3 ans en fonction du traitement de la tumeur primitive

	Radium	Iridium	Pas d'endocurie	Total
T1	14/17	1/2	1/1	16/20
T2	37/90	13/27	0/2	50/119
(T2a)	(21/44)	(4/7)		(25/51)
(T2b)	(16/46)	(9/20)	(0/2)	(25/68)
T3	14/32	32/66	0/8	46/106
(T3a)	(11/22)	(18/32)	(0/2)	(29/56)
(T3b)	(3/10)	(14/34)	(0/6)	(17/50)
Total	65/139	46/95	1/11	112/245

le pronostic (si l'on met à part les T1, le pronostic des N0 est identique à celui des N1 et des N2, pour T2 et les T3).

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	Radium	Iridium
Taux bruts	42 %	48 %
Taux pondérés	40 %	50 %

Les taux pondérés sont obtenus en supposant que tous les malades (T2a, T2b, T3a, et T3b) ont été traités soit par radium, soit par iridium 192.

Trois faits apparaissent (1) la proportion des curages negatifs (N—) est relativement importante (39/129 soit 30 %) (2) la repartition et la survie des N+ et des N— est sensiblement equivalente entre les Np1 et les Np2 (3) le pronostic des curages negatifs (N—) est bon et s identifie a celui des N0 restes N0 74 % de survie a 3 ans pour les N— et 72 % pour les N0 restes N0

Parmi les curages positifs (N+) nous avons etudie le potentiel extensif des metastases ganglionnaires en observant l etat de la capsule enveloppant les ganglions metastatiques Nous avons regroupe les cas avec capsule intacte (C—) et ceux avec capsule envahie ou rompue (C+) par le processus epitheliomateux En voici la survie a 3 ans

N +	C +		C –		Total	
Np1	2/25	(8 %)	14/29	(48 %)	16/54	(29 %)
Np2	6/27	(22 %)	6/9	(66 %)	12/36	(33 %)
Total	8/52	(15 %)	20/38	(53 %)	28/90	(31 %)

Deux faits apparaissent

1 La proportion des capsules rompues est legerement minoritaire parmi les Np1 (25/54 46 %) elle devient fortement majoritaire parmi les Np2 (27/36 75 %) La frequence des capsules rompues (C+) s accroit donc avec le temps

2 Le pronostic des curages positifs (N+) est fondamentalement determine par l etat de la capsule il est tres sombre parmi les cas avec capsule rompue (15 % de survie a 3 ans) avec cependant un meilleur pronostic parmi les Np2 il reste bon parmi le cas avec capsule intacte (53 % de survie a 3 ans) avec un taux legerement superieur a la moyenne de survie globale (53 %) contre 46 %)

Nous avons enfin cherche a apprecier le role de la teleradiotherapie (tele roentgentherapie sous 200 kV ou telecobalt) des aires lymphatiques sur la survie a 3 ans Parmi nos 90 malades avec curage positif (54 Np1 + 36 Np2) un certain nombre ont subi une irradiation lymphatique superieure a 5 000 rad (54 cas) cependant que les autres n ont pas ete irradies (surtout parmi les Np2) ou ont subi une irradiation inferieure a 4 500 rad (36 cas) Les resultats de la survie a 3 ans pour ces deux groupes sont les suivants

Teleradiotherapie de 0 a 4 500 rad	7/36	(19 %)
Teleradiotherapie plus de 5 000 rad	21/54	(39 %)

Il apparait une survie deux fois plus importante pour le groupe des malades ayant subi une irradiation lymphatique superieure a 5 000 rad Il faut noter que la proportion d adenopathies avec capsule rompue (C+) est identique dans chaque groupe

rents sont basés sur des données préthérapeutiques Il convient de les reprendre en fonction de l'évolution clinique et thérapeutique

Deux groupes sont à envisager les N0 d une part, les Np d autre part (c e t a dire les ganglions palpables (p) rassemblant les N1, N2 et N3)

Sur une durée d observation de 3 ans, la moitié des N0 sont devenus Np 67/134

Les Np se répartissent donc en 111 malades avec adénopathie d emblée (Np1) et 67 malades avec adénopathie secondaire (Np2), soit, au total, 178 malades 178/245 = 73 %

Parmi les malades avec adénopathie d emblée (Np1), 83/111 ont subi un curage ganglionnaire, soit 75 %, parmi les malades avec adénopathie secondaire (Np2), 46/67 ont subi un curage ganglionnaire, soit 68 % Les indications chirurgicales ont donc été réalisées dans une proportion équivalente pour les Np1 et les Np2

La survie à 3 ans pour ces différentes catégories s établit de la façon suivante

N0 restés N0	48/67	72 %
Np1 opérés	39/83	47 %
Np2 opérés	18/46	39 %
Np1 non opérés	5/28	47 %
Np2 non opérés	2/21	10 %

On constate que la survie à 3 ans et très élevée pour les malades re tés sans adénopathie (treize T1, douze T2a, dix huit T2b quatorze T3a, dix T3b), elle est proche de la moyenne générale pour les Np opérés avec un pronostic un peu plus sévère pour les Np2 (cinq T1, onze T2a, quinze T2b, neuf T3a six T3b) que pour les Np1 (un T1, dix huit T2a vingt trois T2b, vingt deux T3a, dix neuf T3b), elle est très faible pour les Np non opérés, avec un pronostic lui aussi plus sévère pour les Np2 (nul T1, sept T2a, cinq T2b, quatre T3a, cinq T3b) que pour les Np1 (un T1, trois T2a, sept T2b, sept T3a, dix T3b) On constate en outre, que la proportion des adénopathies (Np1 et Np2) est identique pour les T2 et les T3 (89/119 T2 et 82/106 T3)

Parmi les 129 malades ayant subi un curage ganglionnaire (83 Np1 + 46 Np2), 90 présentent à l'examen histologique une ou plusieurs métastases ganglionnaires (N+ curage positif), 39 ne présentent que des ganglions sains (N— curage négatif) En voici la survie à 3 ans, sur un tableau plus détaillé

	N +		N −		Total	
Np1	16/54	(29 %)	23/29	(79 %)	39/83	(47 %)
Np2	12/36	(33 %)	6/10	(60 %)	18/46	(39 %)
Total	28/90	(31 %)	29/39	(74 %)	57/129	(44 %)

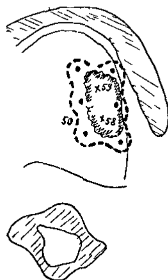


Fig 3 Schema de dosimetrie etabli par la technique sur points Plan trans ersal Dose de base 59 et 58 rad/heure pour 1 mCi/cm Dose de reference (isodose en pointille) 50 rad/heure

de survie a 3 ans entre malades (T2 et T3) traites par radium ou iridium 192 (respectivement 40 % et 50 %) a ete etablie des notre premier controle de denombrement avec 50 dossiers cette difference n a pas varie (a l unite pres) lors des denombrements ulterieurs (100 150 200 et finalement 245 dossiers) Cela prouve selon nous la possibilite de tirer des conclusions compara tives valables sur de petits nombres dans le cadre tres particulier des carcinomes epidermoides de la cavite buccale

La morphologie histo-pathologique de nos 245 cas est remarquablement homogene 93 % de nos cas sont des carcinomes differencies contre seulement 7 % de formes peu ou pas differenciees (18 cas faiblement differencies trois T1 huit T2 sept T3)

La faible proportion de recidives au niveau de la tumeur primitive parmi les cas traites par iridium 192 est manifestement en relation avec les progres techniques de ces epingles (technique par gouttieres vectrices) ou fils (technique par tubes plastiques) radio actifs Ces progres sont lies a trois facteurs prin cipaux (1) la preparation non radio active qui permet d obtenir une mise en place rigoureuse et controlee du dispositif radifere (2) la continuite en toutes mesures des lignes radio actives qui permet d envelopper largement le volume cible a l interieur d une isodose de reference couvrant regulierement la surface muqueuse et les plans profonds du muscle lingual (figures 2 3 4) (3) notre systeme relationnel de dosimetrie avec son controle par la technique sur points qui permet de deceler avec precision d eventuels points

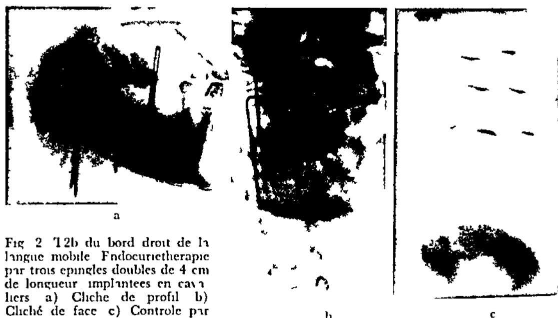


Fig. 2. T2b du bord droit de la langue mobile. Endocuriethérapie par trois épingles doubles de 4 cm de longueur implantées en cavaliers. a) Cliché de profil. b) Cliché de face. c) Contrôle par tomographie transversale.

Discussion

Nous avons inclu dans cette même étude les épithéliomes de la langue mobile et ceux du plancher parce qu'il s'agit de la même famille tumorale. La plupart de ces carcinomes épidermoïdes naissent autour du sillon pelvi-lingual qu'il s'agisse du bord de langue ou du plancher. Dans la mesure où l'on peut distinguer un épithélioma du plancher de celui de la langue mobile, nos contrôles de dénombrement ne nous ont montré aucune divergence tant au niveau de la clinique que du traitement et des résultats.

Nous avons éliminé les T4 de notre travail (tumeurs de plus de 5 cm de longueur et de plus de 3 cm d'infiltration), d'une part parce que notre protocole thérapeutique est différent et moins systématique (l'endocuriethérapie n'a été appliquée que de façon occasionnelle) au niveau de ces grosses lésions, d'autre part parce que la plupart des T4 débordent largement les limites de la langue mobile et du plancher et ne peuvent plus être regroupés dans le cadre exclusif de ces structures.

Le caractère rétrospectif de notre étude d'une part, la subdivision de nos différentes catégories cliniques en de petits groupes cliniques d'autre part, ne nous ont pas permis de tirer de nos résultats des conclusions statistiquement rigoureuses. Il n'en reste pas moins que l'homogénéité clinique et biologique des malades atteints de carcinomes épidermoïdes de la langue mobile et du plancher permet, selon nous, de tirer des conclusions significatives à partir de faibles différences entre de petits groupes. C'est ainsi que la différence de 10 %



Fig 3 Schema de dosimetrie etabli par la technique sur points Plan transversal Dose de base 59 et 58 rad/heure pour 1 mCi/cm Dose de reference (isodose en pointille) 50 rad/heure

de survie a 3 ans entre malades (T2 et T3) traites par radium ou iridium 192 (respectivement 40 % et 50 %) a ete etablie des notre premier controle de denombrement avec 50 dossiers cette difference n a pas varie (a l unite pres) lors des denombrements ulterieurs (100 150 200 et finalement 245 dossiers) Cela prouve selon nous la possibilite de tirer des conclusions comparatives valables sur de petits nombres dans le cadre tres particulier des carcinomes epidermoides de la cavite buccale

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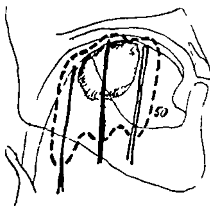


Fig. 4. Schéma de dosimétrie en vue de profil. On retrouve l'isodose de référence en pointillé de 50 rad/heure.

froids ou points chauds et de les corriger par des variations dans la durée d'irradiation de telle ou telle ligne radio-active.

Le pronostic de ces récidives au niveau de la tumeur primitive reste cependant relativement favorable. C'est ainsi que sur 30 récidives (radium ou iridium) qui ont pu être reprises par un traitement à visée curatrice, on constate à 3 ans treize guérisons locales stables (sept guérisons obtenues par une nouvelle application d'endocuriethérapie, six guérisons obtenues par une exérèse chirurgicale).

Nous constatons 85 cas de nécroses (dont 46 cas n'intéressant que les tissus mous et 35 cas intéressant l'os mandibulaire associé ou non aux tissus mous) parmi les 234 cas traités par endocuriethérapie, soit 36 % de nécroses. La plupart de ces nécroses surviennent entre 3 et 12 mois après l'endocuriethérapie. Dans 28 cas (soit 33 % du total des nécroses) une hospitalisation a été nécessaire du fait de l'infection, des douleurs et, par voie de conséquence, de la dénutrition.

Dans les 57 autres cas (67 % du total des nécroses), il ne s'agissait que de radio-lésions limitées et régressives sans retentissement général ou fonctionnel important.

La plupart de ces nécroses sont restées isolées : nous ne constatons que 10 cas associant nécrose et récidive (quatre T2 et six T3). Aucun cas de décès n'a pu être imputé à la seule évolution de la nécrose. Deux faits intéressants sont à souligner :

1. La relation entre le nombre progressif de lignes radio-actives implantées (c'est-à-dire l'importance du volume traité) et la fréquence des nécroses :

	1–2	3–4	5–6	7–8	9–10
Nécroses	9 %	13 %	26 %	41 %	56 %

Cette relation explique, en partie, la plus grande fréquence des nécroses parmi les cas traités par iridium 192 (49/95 avec iridium 192 contre 36/139 avec dium).

2 L absence de relation entre la duree de l irradiation et la frequence des necroses (pour une meme dose tumorale et dans les limites de nos etalements) entre 3 et 9 jours de duree d irradiation la frequence des necroses reste la meme

Le pronostic de ces necroses reste finalement favorable nous constatons parmi les T2 et les T3 traites par endocurietherapie 60 % de survie a 3 ans pour les cas avec necrose contre 34 % pour les cas sans necrose

L appreciation clinique de l envahissement metastatique des ganglions reste tres incertaine Ceci est particulierement vrai pour les epitheliomas de la cavite buccale ou l on constate dans la grande majorite des cas des ganglions palpables dans la region sous-maxillaire sans qu il soit possible — quelle que soit leur taille — d affirmer s il s agit de ganglions inflammatoires de sous maxillites ou de metastases Dans 30 % des cas rappelons le, qu il s agisse de Np1 ou de Np2 l histologie apres curage chirurgical s est revelee *negative* (N—) Cette discordance frequente entre la clinique et l histologie prouve le *caractere* hasardeux de notre appreciation clinique La question se pose donc de savoir s il faut ou non recommander un curage chirurgical systematique des aires ganglionnaires satellites qu il y ait ou non des ganglions cliniquement suspects l identité du pronostic des Np1 et des Np2 histologiquement N+ ne permet pas dans les limites de notre travail de repondre a cette question seul un essai therapeutique statistiquement controle, permettrait d y repondre (cet essai est actuellement en cours d etude a l Institut Gustave Roussy)

Parmi les onze malades qui *n ont* pas *subi* d endocurietherapie un seul a ete traite par electro-chirurgie il s agissait d un T1 N0 actuellement gueri a plus de 9 ans Les dix autres (deux T2b deux T3a et six T3b) ont tous présenté une poussee evolutive avant la date fixee pour l endocurietherapie et n ont ete traites que par teleradiotherapie a noter que l un d entre eux (T3b N0) est gueri depuis plus de 9 ans apres 5 000 rad (telecobalt) tous les autres sont decedes en quelques mois

Il reste a discuter les causes de deces a 3 ans

Dix malades sont morts sans que nous ayons pu obtenir de precision sur la cause de leur deces

Quarante sept malades sont morts avec une recidive au niveau de la tumeur primitive dont vingt huit avec une recidive ganglionnaire associee, cinq avec des lesions a distance associees (metastases autres cancers ou maladies intercurrentes) quatorze avec une recidive linguale ou pelvienne isolee Parmi ces quatorze cas on denombre un T2a (traite par radium) cinq T2b (tous traites par radium), deux T3a (dont un traite par radium l autre traite par iridium) six T3b (dont deux traites par radium et quatre traites par iridium)

Soixante-dix malades sont morts avec une recidive ganglionnaire cervicale,

dont vingt huit, déjà cités, avec une récidive de la tumeur primitive associée, dix sept avec des lésions à distance associées, vingt cinq avec une récidive ganglionnaire isolée. Les adénopathies sont une des causes principales de décès.

Vingt cinq malades sont morts avec des métastases, dont quatorze avec une récidive buccale ou ganglionnaire (deux buccales, douze ganglionnaires), onze avec des métastases isolées ou associées à d'autres lésions à distance. A noter la prédominance des métastases médiastinales ou pulmonaires (quinze cas).

Quinze malades sont morts avec d'autres cancers, dont quatre avec une récidive buccale ou ganglionnaire et onze isolées ou associées à d'autres lésions à distance. Tous ces autres cancers se situaient au niveau des voies aérodigestives supérieures (quatre oro-pharynx, quatre hypopharynx, quatre oesophages, deux bronches), sauf un cas (rectum).

Vingt trois malades sont morts d'une affection intercurrente, dont cinq avec une récidive buccale ou ganglionnaire, et dix huit isolées ou associées à d'autres lésions à distance. *La plupart de ces affections intercurrentes étaient liées* à des complications d'un éthylisme chronique.

Au total, les causes de décès sont largement déterminées par l'évolution de lésions à distance, associées ou non à des récidives buccales ou ganglionnaires. Si l'on met à part les cas avec métastases associées à des récidives buccales ou ganglionnaires (dont l'apparition est peut être liée à la non stérilisation du processus loco-régional), on totalise finalement quarante cinq cas où le décès est inexorablement lié au développement de lésions à distance. On peut donc constater que 34 % des décès sont en relation inexorable avec des lésions à distance, 18 % de l'ensemble de nos malades meurent donc, avant 3 ans, de causes indépendantes de la seule évolution de la tumeur loco-régionale (tumeur primitive ou adénopathies cervicales).

Nous n'ouvrirons pas de discussion avec les autres travaux sur le traitement des cancers de la langue mobile ou du plancher buccal pour la raison fondamentale qu'aucune comparaison significative n'est possible entre des travaux où la classification TNM n'a pu être préalablement établie en commun : autant d'auteurs indépendants, autant de classifications différentes (Fletcher et coll. 1962, Saxena et coll. 1967).

Conclusion

Parmi 245 malades atteints de carcinomes épidermoïdes de la langue mobile ou du plancher buccal (vingt T1, cent dix neuf T2, et cent six T3), la comparaison entre les cas traités par endocuriethérapie avec radium (dosimétrie empirique) et ceux traités par iridium 192 en préparation non radio active

(dosimetrie controlee) fait apparaitre une considerable difference dans la frequence des recidives (ou non sterilisations) au niveau de la tumeur primitive pour les tumeurs de moins de 4 cm de grand diametre sur un delai d observations de 3 ans la frequence des recidives est de l ordre de 3 % avec l iridium 192, alors qu elle atteint 35 % avec le radium Les nouvelles techniques d endocurietherapie avec preparation non radio-active ont donc reduit les risques de recidive locale a un taux presque negligeable

Le pronostic reste cependant lourdement greve par la frequence des adenopathies cervicales soixante dix de nos malades sont morts avec une recidive ganglionnaire (53 % des deces) Notre protocole de curage chirurgical et de teleradiotherapie postoperatoire a la demande clinique des adenopathies ne montre pas de difference significative dans la survie des malades avec adenopathie primitive ou adenopathie secondaire Les formes histologiques d adenopathies avec rupture capsulaire ont un pronostic particulierement severe

Le pronostic est egalement assombri par la frequence des lesions a distance metastases sans recidive loco-regionale autres cancers maladies intercurrentes Quarante cinq de nos malades sont morts avec des lesions a distance (34 % des deces)

Au total la survie a 3 ans de nos 245 malades est de 46 %

RÉSUMÉ

Parmi 245 malades atteints de carcinomes epidermoides de la langue mobile ou du plancher buccal la comparaison entre les cas traites par endocurietherapie avec radium et ceux traites par iridium 192 en preparation non radio active fait apparaitre une considerable difference dans la frequence des recidives au niveau de la tumeur primitive pour les tumeurs de moins de 4 cm de grand diametre sur un delai d observation de 3 ans la frequence des recidives est de l ordre de 3 % avec l iridium 192 alors qu elle atteint 35 % avec le radium Les nouvelles techniques d endocurietherapie avec preparation non radio active ont donc reduit les risques de recidive locale a un taux presque negligeable Au total la survie à 3 ans de nos 245 malades est de 46 %

SUMMARY

The results of the treatment of 245 patients with epidermoid carcinoma of the tongue or floor of the mouth by means of endocurietherapy with radium or iridium 192 a non radioactive preparation are discussed A considerable difference in the reaction of the primary growth over a three year period was evident With tumours less than 4 cm in diameter the rate of recurrence was of the order of 3 per cent with iridium 192 while it reached 35 per cent with radium These new techniques with non radioactive preparations thus reduce the risk of local recurrence to an almost negligible figure The three year survival rate for the whole material was 46 per cent

ZUSAMMENFASSUNG

Die Ergebnisse der Behandlung von 245 Patienten mit einem epidermoiden Carcinom der Zunge oder des Mundgrundes unter Anwendung der Endocurietherapie mit Radium oder Iridium 192 (einem nicht radioaktiven Präparat) werden besprochen. Ein bedeutender Unterschied in der Reaktion des primären Wachstums während einer Drei-Jahres-Periode war deutlich. Bei Tumoren mit weniger als 4 cm im Diameter war die Recidivfrequenz bei Iridium 192 in der Grössenordnung von 3 %, während sie bei Radium 35 % erreichte. Diese neue Technik mit einem nicht radioaktiven Präparat vermindert somit das Risiko eines Recidivs zu einer nahezu zu vernachlässigenden Grösse. Die Drei-Jahres-Überlebensrate für das gesamte Material betrug 46 %.

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PORTABLE ^{90}Sr ^{90}Y APPLICATOR WITH INTERCHANGEABLE PLASTIC SHUNT FOR EXTRACORPOREAL BLOOD IRRADIATION

by

H SKOLDBORN B ROSENGREN INGER RAGNHULT and R KELLGREN

Extracorporeal irradiation of blood has been used in the *treatment of leu*kaemia (LAJTHA et coll 1968 THOMAS et coll 1965) and for immunosuppression in conjunction with renal transplantations (HUME et coll 1966 MAGINN & BULLIMORE 1968 ROSENGREN et coll 1968 PERSSON et coll 1969)

Repeat extracorporeal blood irradiation requires an arteriovenous shunt as described by SCRIBNER *et coll (1960)* The irradiation units usually utilize gamma rays from ^{60}Co or ^{137}Cs or beta rays from ^{90}Sr ^{90}Y (KUHL et coll 1964 CRONKITE 1967 GILBERT & *LAJTHA 1966* LAJTHA et coll 1968) The use of roentgen rays and ultraviolet light has also been reported (VAUGIER 1969)

A plastic or silicone rubber tube coiled around a specially designed radiation source is usually employed for conducting the blood A conventional cobalt machine may also be used however in which event the highest efficiency will be attained when the coil is suitably *shaped* and introduced into the collimator system close to the radiation source ROSENGREN & SKOLDBORN (1968) applied the latter principle to a Siemens Gammatron III (3 000 Ci ^{60}Co)

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ZUSAMMENFASSUNG

Die Ergebnisse der Behandlung von 245 Patienten mit einem epidermoiden Carcinom der Zunge oder des Mundgrundes unter Anwendung der Endocurietherapie mit Radium oder Iridium 192, einem nicht radioaktivem Präparat, werden besprochen. Ein bedeutender Unterschied in der Reaktion des primären Wachstums während einer Drei-Jahres-Periode war deutlich. Bei Tumoren mit weniger als 4 cm im Diameter war die Recidivfrequenz bei Iridium 192 in der Grossenordnung von 3 % während sie bei Radium 35 % erreichte. Diese neue Technik mit einem nicht radioaktiven Präparat vermindert somit das Risiko eines Recidivs zu einer nahezu zu vernachlässigenden Grösse. Die Drei-Jahres-Überlebensrate für das gesamte Material betrug 46 %.

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total dose may be exceeded too soon The alternative is fractionated irradiation with higher transit doses

A light irradiation unit that the patient can carry on his person day and night has many advantages The supply tubes can be made short and complete heparinization is probably unnecessary in many cases Such an applicator has been described by HUME et coll (1966) and by SUNDBOM et coll (1969), although the average dose rate to the blood was far below the optimum

The aim with the design to be described was to produce the lightest possible unit portable on the patient s upper arm and delivering 2 000 to 4 000 rad to 5 litres of blood per 24 hours The bremsstrahlung dose rate to the most exposed point should not exceed 400 rad per week (at 2 000 rad/24 h to the blood) The irradiation element was to be a readily interchangeable sterilizable plastic shunt capable of withstanding at least a 24 hour continuous use It should be possible to insert and withdraw the shunt without risk and there should be no hot spots when the unit is clo ed

Design and operation data

Radiation source and shielding The prototype in question was developed tep by step in close collaboration with the Radiochemical Centre Amersham Buckinghamshire England The optimum combination of materials in a suitable radiation shield would probably have been beryllium/uranium (depleted ^{38}U) However graphite was substituted for beryllium the dimensions determined being 5 mm graphite nearest the source followed by 2 3 mm uranium The radiation source consists of ^{90}Sr ^{9}Y titanate incorporated in four aluminium foils two on each side of the plastic shunt The active area of each foil is 100 mm × 8 5 mm and the total activity 3 3 Ci A thin layer of stainless steel is interposed between the source and the plastic shunt A 2 5 mm thick aluminium frame used in a previous model was kept in the present model Hence the latter is less than optimal as to low weight and low bremsstrahlung with the 5 mm graphite plus 2 3 mm uranium specified

The design is illustrated in Figs 1 and 2 The unit is housed in a 3 2 mm perspex casing screwed together and open at one end Outside this is a 0 5 mm stainless steel case fitted with an adapter of the same material having a wall thickness of 1 25 mm (see Fig 1) The latter makes insertion or removal of the plastic shunt a rapid and simple operation and at the same time serves as an adequate radiation shield in this direction The total weight including casing and stainless steel adapter is 1 35 kg

Plastic shunt The plastic shunt is depicted in Fig 2 It is moulded of rigid PVC in a series of 1 000 (Kautex Werke Hangelar Siegburg West

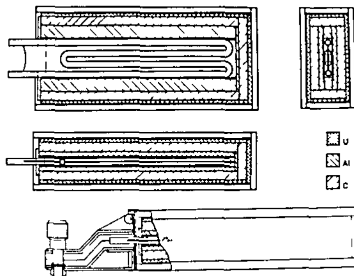


Fig 1 Irradiation unit with plastic shunt in three projections and the assembled applicator with adapter

The requirement for effective radiation shielding makes units employing ^{60}Co or ^{137}Cs sources heavy and bulky, and, moreover, necessitate fairly long tubes Their greatest advantages lie in that they make possible high and variable dose rates and transit doses The specially adapted Siemens Gammatron III enabled an average dose rate (to 4 litres of blood) of 1 300 rad/h and a transit dose of about 600 rad

Irradiation units with ^{90}Sr ^{90}Y can be made lighter than those with ^{60}Co or ^{137}Cs but here too the hard bremsstrahlung makes effective shielding necessary The equipment used by GILBERT & LAJTHA has provisions for a variable as well as a high dose rate It requires, however, rather long tubes and complete heparinization of the patient and must therefore always be operated by skilled staff

A comparatively light irradiation unit with 2 1 Ci ^{90}Sr ^{90}Y (weight including shielding 22 kg) is in use at Sahlgrenska Sjukhuset (ROSENGREN et coll 1968) However, providing merely an average dose rate (to 4 litres of blood) of 100 rad/h and a maximal transit dose of 50 rad, it has primarily been used when relatively small doses have been required, e g in the treatment of lymphatic leukaemia

Owing to the interchange of lymphocytes between the circulating blood and lymphatic tissue the blood must be irradiated continuously for a long time if a high proportion of the lymphocytes are to be affected by the irradiation Hence a comparatively low transit dose may be preferable, or the admissible



Fig 3 The applicator in clinical use

The shunts are first carefully inspected and all with visible defects (including external width under 4 0 mm) are discarded A series of 100 were thus approved and further investigated Their mean weight was 5 23 g with a maximum deviation of 2 % the maximum deviation in eighty of them not exceeding 1 % By weighing the contained water it was found that the average volume (including the non irradiated parts of the shunt about 13 % of the total volume) was 3 49 cm³ Eighty eight of them deviated from the mean by at most 5 % and were approved for clinical use Their standard deviation was 2 1 % The deviation in weight and volume were uncorrelated

A change in weight of 2 % corresponds to a wall thickness change (provided the external measurements are equal) of about 0 01 mm This alter the dose rate to the liquid in the shunt by at the most 2 % The dosage erro is thus mainly a function of volume deviations

If the plastic shunts are selected and approved for clinical use in accordanc with these premises the average dose rate to the blood will be reproducibl within ± 10 % an acceptable value for the clinical applications intended A more comprehensive series of measurements by Fricke dosimetry will never theless be carried out in order to check the conclusions reached

Operation The plastic shunt is sterilized with fluid just like a plast catheter it is coupled to the Scribner shunt by two silicone rubber tubes

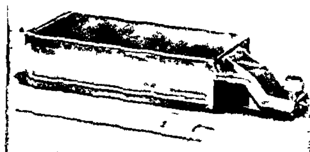


Fig 2 The applicator and a plastic shunt

Germany) The channel in the irradiated portion of the shunt (between the active foils) is approximately 400 mm long and its volume is 3 cm³ The wall thickness is 0 45 to 0 50 mm and its external width 4 mm The cross section is oval with somewhat flattened margins nearest to the active foils It proved impossible to mould a rigid PVC shunt with a rectangular cross section

Average dose rate and transit dose Two methods were used for measurement of the average dose rate to the circulating blood At the first measurement, which was made at Amersham, 40 lithium fluoride dosimeters were uniformly distributed in the irradiated portion of the plastic shunt In the second series of measurements a solution of ferrous sulphate was used as dosimeter A volume of 250 ml of this Fricke solution was pumped through the plastic shunt via the same system of silicone rubber tubes as in clinical practice After correction for oxidation by the tubes and shunt the average dose to the solution could be estimated Repeat measurements with seven shunts yielded a mean dose of 4 25 krad to 5 litres of blood in 24 hours with a maximum deviation of 6 2 % The adopted G value was 15 4 (Fregene 1967)

The value of 4 25 krad/24 h to 5 litres of blood corresponds to an average dose rate in the irradiated part of the shunt (about 3 cm³) of some $2\ 9 \times 10^5$ rad/h or 4 9 krad/min The properties of PVC material deteriorate by some 25 % after irradiation with $1\ 1 \times 10^8$ rad, according to Collins & Calkins (APEX 261 1956) Hence without material damage due to the irradiation, the plastic shunt may be used without risk for at least two weeks continuously, in other words longer than it would ever be used in clinical practice (15 days corresponds to 63 krad to 5 litres of blood)

The flow velocity in the shunt in clinical practice was determined to about 180 cm³ per minute, corresponding to a transit dose of 80 rad

Accuracy If the patient s blood volume is known, the accuracy of the dosage will depend mainly on the properties of the plastic shunt (variations in wall thickness and volume)



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Operation The plastic shunt is sterilized with fluid just like a plastic catheter it is coupled to the Scribner *shunt* by two silicone rubber tubes so

that the applicator can be attached to the upper arm The shunt is then introduced into the irradiation unit, a rapid procedure entailing no risks whatsoever from radiation aspects if properly done It is shown in Fig 3 how the applicator is mounted on the patient's upper arm

Radiation shielding The absorbed surface dose rates at the centre of the top and bottom sides of the fully enclosed applicator were 3 5 rad/h Accordingly, the dose to the most exposed point on the upper arm will not exceed 400 rad at an average dose of 20 000 rad to 5 litres of blood This roughly agrees with the premises stated in the introduction Obviously the dose to other parts of the body will be much lower The following dose rates have been measured with lithium fluoride dosimeters axilla 225, nape of neck 15, sternum 50 and groin 25 mrad/h

The dose rate at a distance of 1 m from the top or bottom of the applicator is 5 mrad/h but less in other directions Staff and others should thus not be unnecessarily in the patient s immediate vicinity With this rule observed no problems should arise Radiation doses to members of the staff are at present checked by film dosimeters

Clinical use The blood irradiation applicator was ready for clinical use in July 1969 and so far (October 1969) it has been used in only five patients for the purpose of immunosuppression prior to kidney transplantation Owing to its interchangeable plastic shunt and convenient dimensions it is easy to apply and appears to cause a minimum of inconvenience to the patient

We have up to the present been reluctant to waive heparinization as a protection against coagulation in the irradiation unit Experience indicates that the most critical points are the attachments to artery and vein Yet, irradiation without heparinization will be tried and we contemplate an average dose of at least 20 000, at the most about 30 000 rad, before renal transplantation is envisaged

The brief observation period prevents any prediction concerning the long term immunosuppressive effects in these cases

Development prospects As mentioned before the applicator might be improved A minimum of aluminium around the irradiation source would reduce the intensity of the bremsstrahlung Any beta radiation not absorbed in the shunt with contained blood will then be largely stopped by graphite (atomic number 6) instead of, as now, by aluminium (atomic number 13) and graphite Simultaneously the irradiation unit would become smaller and lighter with uranium plates of the same thickness If beryllium (atomic number 4) were used in place of graphite there would be a further gain, although the applicator would then become much more expensive If the radiation source could be made

linear with the same outline as the plastic shunt it would probably be feasible to increase the dosage yield per Ci ^{90}Sr ^{90}Y

The effectiveness of the irradiation unit described is around 13 % i e the blood circulating in the shunt absorbs 13 % of the energy released at the source (26 % if only the radiation emitted into the solid angle 2 π is considered)

The average dose rate to the blood may be affected significantly by changes either in the activity of the source or in the shunt volume The shunt described was designed with great attention to the risk of blood coagulation This hazard was deemed greater in a flat irradiation element which moreover would greatly affect the transit dose owing to differences in blood velocity through a rectangular cross section Experiences with the applicator will act as a guide in the design of its successors

SUMMARY

A portable ^{90}Sr ^{90}Y applicator for extracorporeal irradiation of blood providing an average dose rate of 4 3 krad/24 h per 5 litres is described Accuracy of dosage and radiation hazards are discussed It is likely that the applicator will be used for immunosuppression and in the treatment of leukaemias

ZUSAMMENFASSUNG

Eine transportable Apparatur fur extracorporale Bestrahlung des Blutes mittels ^{90}Sr ^{90}Y mit einer Intensitat von 4,3 krad/24 Stunden pro 5 Liter wird beschrieben Die Genauigkeit der Dosierung und die Bestrahlungsgefahren werden diskutiert Anwendungsmoglichkeiten fur die Methode sind die Leukamien und die Unterdruckung der Autoimmunisationsvorgange

RÉSUMÉ

Description d un applicateur portatif de ^{90}Sr ^{90}Y pour l irradiation extracorporelle du sang delivrant un debit de dose moyenne de 4 3 krad/24 heures et par 5 litres Les auteurs etudient la precision la dosimetrie et les dangers d irradiation Il est vraisemblable que cet applicateur sera utilise pour l immunosuppression et dans le traitement des leucoses

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that the applicator can be attached to the upper arm The shunt is then introduced into the irradiation unit, a rapid procedure entailing no risks whatsoever from radiation aspects if properly done It is shown in Fig 3 how the applicator is mounted on the patient's upper arm

Radiation shielding The absorbed surface dose rates at the centre of the top and bottom sides of the fully enclosed applicator were 3 5 rad/h Accordingly, the dose to the most exposed point on the upper arm will not exceed 400 rad at an average dose of 20 000 rad to 5 litres of blood This roughly agrees with the premises stated in the introduction Obviously the dose to other parts of the body will be much lower The following dose rates have been measured with lithium fluoride dosimeters axilla 225, nape of neck 15, sternum 50 and groin 25 mrad/h

The dose rate at a distance of 1 m from the top or bottom of the applicator is 5 mrad/h but less in other directions Staff and others should thus not be *unnecessarily in the patient s immediate vicinity With this rule observed no* problems should arise Radiation doses to members of the staff are at present checked by film dosimeters

Clinical use The blood irradiation applicator was ready for clinical use in July 1969 and so far (October 1969) it has been used in only five patients *for the purpose of immunosuppression prior to kidney transplantation* Owing to its interchangeable plastic shunt and convenient dimensions it is easy to apply and appears to cause a minimum of inconvenience to the patient

We have up to the present been reluctant to waive heparinization as a protec tion against coagulation in the irradiation unit Experience indicates that the most critical points are the attachments to artery and vein Yet, irradiation without heparinization will be tried and we contemplate an average dose of at least 20 000, at the most about 30 000 rad, before renal transplantation is envisaged

The brief observation period prevents any prediction concerning the long term immunosuppressive effects in these cases

Development prospects As mentioned before the applicator might be improved *A minimum of aluminium around the irradiation source* would reduce the intensity of the bremsstrahlung Any beta radiation not absorbed in the shunt with contained blood will then be largely stopped by graphite (atomic number 6) instead of, as now, by *aluminium (atomic number 13) and graphite* Simultaneously the irradiation unit would become smaller and lighter with uranium plates of the same thickness If beryllium (atomic number 4) were used in place of graphite there would be a *further gain, although the applicator* would then become much more expensive If the radiation source could be made

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EVALUATION OF REGIONAL LYMPH DRAINAGE FROM MAMMARY GLAND AND HAND TO AXILLARY NODES

by

A HULTBORN L HULTEN B ROOS M ROSENCRANTZ B ROSENGREN and CH AHREN

The lymphatics of the breast used to be outlined anatomically by post mortem injection studies Indirect evidence of the direction of the lymph flow was also obtained in examinations of the sites of metastases associated with carcinoma of the breast In recent years more delicate techniques have been adopted to investigate the lymphatic drainage Thus local injection of dyes and radioactive isotope tracers in the mammary gland have been used these are transported to the regional nodes by the natural lymph flow (HULTBORN & JONSON 1955 HULTBORN et coll 1955 1955 and TURNER WARWICK 1959) Furthermore direct lymphography from the dorsum of the hand has made it possible to demonstrate the lymphatics and lymph nodes in the axilla this method has been evaluated in the diagnosis of lymph node metastase (KENDALL et coll 1963 HULTEN et coll 1966) The latter method has however been criticized because it cannot be taken for granted that the lymph flow from the arm and breast is diverted to the same group of axillary lymph nodes This is of surgical interest

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(1966) Patent blue was injected subcutaneously and interdigitally leading to blue staining of the lymph vessels of the dorsum of the hand One of the vessels was exposed and cannulated with a fine bore polythene catheter, the contrast medium being Lipiodol Ultra Fluide 38 % An automatic injection pump was used for the injection at a rate of 0 05 to 0 1 ml/minute of 4 to 7 ml of the medium which was heated to around 50° C to reduce its viscosity

One minute after the start of the injection, objective verification of the intra lymphatic injection was obtained by roentgen examination The axillary and supraclavicular regions were examined immediately after the contrast medium injection A further roentgen examination was made up to about 24 hours later, as the lymph nodes could be more readily examined when the lymph vessels had been emptied of their contrast medium content

C *Operative procedures* Radical mastectomy was mostly performed the day after lymphography In order to facilitate subsequent topographic orientation of the whole specimen, and localization of the nodes in the axillary fat silver clips or steel wire sutures were affixed in positions corresponding to the axillary vein and the thoracic wall The axilla was examined roentgenographically after the lymph node dissection and any lymph nodes containing Lipiodol that had been overlooked were identified and removed As may be seen from Fig 2a the lymph nodes in the axilla contained Lipiodol before mastectomy while after *mastectomy* (Fig 2b) no contrast medium remained in the axilla but lay in the lymph nodes in the supraclavicular region which was not included in the operation field

D *Roentgen examination of the specimen* Fine grain double coated double wrapped industrial film Kodak Crystallex was used The breast the axillary specimen and the separately removed fatty *tissue* and lymph nodes were placed on the film either directly or, to prevent wetting protected by thin absorbent paper

The roentgen source in all but one case was a Siemens Pantix tube (type P 125 30 50) the smaller focus measuring effectively 1 2 mm × 1 2 mm was employed The exposure data were focus-film distance 80 cm 320 mA 35 to 40 kV exposure time 2 5 *to* 4 *seconds* The specimens of one of the later cases were examined with the CGR Senographe apparatus made especially for soft tissue radiography and having an effective focus of 0 7 mm × 0 7 mm The exposure data in this case were *focus*-film distance 25 cm 25 mA 25 to 30 kV exposure time 2 seconds

The films were developed in a Pako XM film processor with Gevaert 137 developer for 3 minutes at 21° C

The detail of a roentgenogram is affected by the radiographic contrast and

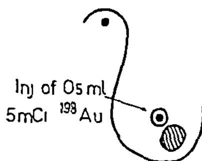


Fig. 1. Carcinoma of the lower medial quadrant of the breast. The arrow indicates the site of injection of radioactive gold craniolateral to the nipple. Palpable node in the axilla.

A method has been worked out for assessing the distribution of the lymph from the arm and the breast and to investigate whether the lymph drainage from these two areas is separate in the axilla. This includes injection of a tracer in the breast combined with lymphography from the dorsum of the hand and subsequent *identification of the axillary lymph nodes in the operation specimen*. A description of the method is given in the present paper, results and clinical implications will be discussed in forthcoming publications.

Investigation procedures

Patients with clinical, roentgenologic and cytologic evidence of carcinoma of the breast but without clinical signs of metastases in the axilla were examined. The procedures were as follows: injection of a tracer substance (^{198}Au) into the breast, lymphography with Lipiodol, radical mastectomy and postoperative investigation of the specimen, including roentgen examination to demonstrate the Lipiodol, measurement of radioactivity, autoradiography and finally histologic examination.

There are two methods of demonstrating the Lipiodol: roentgen recording and histologic examination, and two indicators for radioactivity: direct measurement and autoradiography.

A. Tracer method (^{198}*Au*). The injections of a colloidal suspension of metallic ^{198}Au (GCS 1P and GCS 4P, the Radiochemical Centre, Amersham, England) were made with a fine bore needle into the parenchyma (Fig. 1). A volume of 0.5 to 1.5 ml and an activity of 1.5 to 5.0 mCi were used. The particle size ranged up to 200 Å (GCS 1P) in two cases and 200 to 300 Å (GCS 4P) in six cases. No external counting was made.

B. Lymphography. This was performed next day. The reason for this sequence was that Lipiodol may *impede the introduction of* ^{198}*Au*. *The technique* was that described by KINMONTH (1954), WALLACE et coll. (1961) and HULTEN et coll.



Fig 3 Specimen lymphadenogram

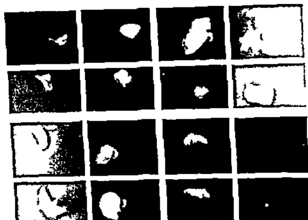


Fig 4 Sample lymphadenograms with a se ies of l mph nodes d ssected out

residual lymph nodes were then dissected out The original location of some of these nodes found at the econd or third stage of dissection could not be estimat ed and these nodes were recorded as from an unknown region

The breast and pectoral muscles were investigated and great care was taken in searching for the nodes dorsally on the major pectoral muscle including the inter pectoral nodes

The magnifying glass mentioned earlier was used in the dissection of the pecimens

An almost exact anatomic location of the major part of the lymph nodes in the axillary fat as well as of those removed separately was possible by detailed orientation during the operation The individual nodes could be identified and transferred to an anatomical diagram and for practical purposes arranged in conventional groups



a b

Fig 2 a) Lymphadenogram before radical mastectomy Axillary and supraclavicular lymph nodes containing Lipiodol a few lymph vessels and extravasated contrast medium in the axilla b) Examination after radical mastectomy with all the axillary nodes containing Lipiodol removed but with supraclavicular nodes beyond the operation field

definition, which in turn are governed by many factors the discussion of which is beyond the scope of this paper The films obtained with the technique described generally had good detail and demonstrated clearly the lymphographic structure when examined through a lens (+ 5 75 diopters, focal length 17 4 cm, diameter 11 7 cm), even in nodes not larger than 1 mm Small lymph nodes not carrying the contrast medium were also sharply outlined

With guidance of the detailed roentgenogram (specimen lymphogram, Fig 3) the lymph nodes were finally dissected out from the axillary fat The individual lymph nodes were mapped out, numbered and arranged in series for individual roentgen examination, using the kind of industrial film referred to above (Fig 4) *The specimens, which were kept in formalin, should then be as dry as possible* since the formalin after penetrating the envelope dissolves the coating of the film (Fig 5)

A problem encountered during the roentgen examinations was that the intensity of the radiation emitted from the ^{198}Au in the *lymph* nodes *sometimes* blackened the film to such an extent that the images of the nodes were partly or even totally obscured (Fig 6a) This could be avoided by increasing the specimen film distance thereby decreasing the radiation intensity in the film plane according to the inverse square law A small increase of 1 5 mm was found to be enough and with a focus film distance of 80 cm did not impair the definition of the roentgenogram to a noticeable degree (Fig 6, b and c)

The remaining axillary fat was always re examined roentgenologically and any

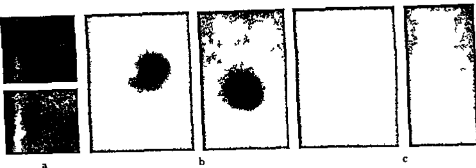


Fig 6 a) The lymph node in the lower image is small but easily identified by its Lipiodol content Section in upper image had high radioactivity blackening the film and obscuring the node (see also fig 4) b) and c) Roentgenograms of small lymph nodes with respectively Lipiodol and high radioactivity The specimens corresponding to (b) were placed directly on the film envelope while for those corresponding to (c) the distance film specimen was increased by 1 5 mm which was sufficient to prevent blackening

G Histologic technique The dissected lymph nodes were fixed in 10 % neutral formaldehyde solution for 12 to 18 hours and routinely prepared for paraffin embedding The blocks were cut at 4 μ and primarily two to four sections were obtained from each block and mounted on slides. This procedure always took place immediately after the operation to allow autoradiography within two and a half days after the dissection of the nodes The sections used for autoradiography were then stained by the van Gieson method for histologic examination for signs of contrast medium deposition of ^{198}Au or existence of metastases Further sections at deeper levels were taken from the paraffin blocks when the specimens proved radioactivity but no evidence of activity was found at autoradiography and no lymph nodes could be demonstrated in the stained sections

Discussion

Demonstration of Lipiodol Only vacuoles or irregular empty cavities remained after the droplets or lakes of Lipiodol had been dissolved during the histotechnical procedure Most nodes had contained large amounts of the medium to give the section a characteristic honeycomb structure (Figs 7 and 8) Even some destruction of the parenchyma had occurred in heavily loaded nodes

Moderate amounts of the contrast medium when present were confined to the sinusoids of the node and could usually be easily identified Difficulties can arise however in differentiating Lipiodol vacuoles from lobules of fat cells or single fat cells which are common as an atrophic phenomenon in axillary lymph nodes



Fig 5 Halo around a lymph node caused by the dissolving action of the formalin on the film

When this part of the investigation was terminated, all lymph nodes and the axillary fat were kept for further quantitative measurements of radioactivity, autoradiography and histologic examination

E Quantitative measurements of radioactivity After dissection of the specimens, each lymph node and the axillary fat were placed separately in a Picker Autowell II Sample Changer, having a well crystal, 3 inches in diameter The lower level discriminator was set at 350 keV and the channel width was 130 keV The count rates ranged from less than one hundred cpm to several hundred thousand cpm All the samples were first measured for 6 seconds and specimens *with low activity (less than 100 counts in 6 seconds)* were then measured again for one minute This method is most sensitive High activity in the remaining axillary tissue indicated the probable presence of any overlooked lymph nodes, and such a specimen was redissected

F Autoradiography Five to eight days after injection of the radioactive gold into the breast, autoradiographs of the histologic sections of the lymph nodes were made according to a simple contact method The same sections were then stained and examined histologically This technique was considered adequate for the purpose because of the high sensitivity of the quantitative measurements in the Picker Autowell II Sample Changer

We used an ordinary roentgendiagnostic cassette and Agfa Gevaert Curix RP film When the cassette was closed, it pressed the film against the histologic sections Two or three series of sections from different levels were selected, and the exposure times were from 24 hours to one week A suitable level of activity is necessary for good results — if blackening of the film was too dense and unsharp the autoradiographic procedure was repeated after a few days, when the isotope had decayed (Fig 7) With the use of this method it is of course important that the section in the paraffin block should really lie at the level of the lymph node If this is not the case, one of the measurements of radioactivity would be lost namely the autoradiographic one This occurred in a few lymph nodes of very small size

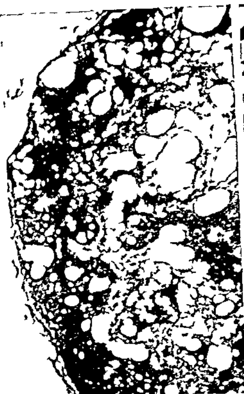


Fig 8 Part of lymph node with signs of Lip odol storage Numerous round o ovoid acuoles through the parenchyma The larger droplets tend to coalesce into lakes of ir regular shape van Gieson × 30



Fig 9 Part of lymph node with marked fat in olvement (lower half) and a few Lipiodol vacuoles (+) between the fat cells It may be noted that the fat cells are of polygonal shape and relati ely uniform s ze van Gieson × ₋10

oids and the difficulty lay in differentiating the vacuoles from artefacts The latter could sometimes be correctly identified when they were connected with other artefacts such as rifts furthermore they often had an irregular shape compared to the spherical Lipiodol droplets The true nature was sometimes in doubt when the slides had been prepared and stained for the demonstration of carcinoma metastases

The nodes in five cases were exposed to Lipiodol for only about 24 hours before fixation in formalin and in these cases there were no inflammatory reactions around the droplets of contrast medium except a few eosinophilic granulocytes in occasional nodes If the experimental model allows exposure for e g 48 hours numerous leukocytes and with even longer exposure many foreign body giant

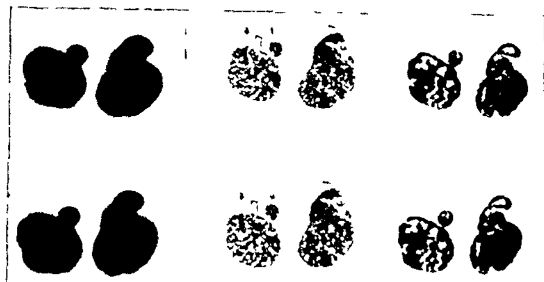


Fig. 7. Autoradiograms exposed 12—15 July to the left and those exposed 15—16 July to the right, in the centre the histologic sections.

(Fig. 8). The distinction may, however, with some experience generally be made. The Lipiodol vacuoles vary considerably in size and tend to coalesce into small lakes. The size of the fat cells is much more constant, and these cells often form small lobules protruding from the hilus of the node. Even if occasional fat cells may have the shape of empty spheroids, like the Lipiodol vacuoles, they usually are somewhat polygonal (Fig. 9).

The cell membrane or traces of the membrane may often be identified, although this may sometimes be difficult or even impossible. However, the employment of thicker sections of about 10 μ or more would much increase the likelihood of identifying the cell membrane or its residues in doubtful specimens. It would then be necessary to cut consecutive sections from the same block at 4 μ to investigate e.g. carcinoma metastases. The fat cell nucleus was usually hard to identify against the numerous parenchymal cell nuclei of the node. A thorough study of the sinusoids and the adjacent lymph vessels, where the Lipiodol vacuoles could be easily identified, was of great value, as was a comparison with the cells of the surrounding fat tissue. With these characteristics, signs of contrast storage, if present to a moderate degree, can always be demonstrated histologically in sections stained by van Gieson or hematoxylin eosin, i.e. methods suitable for the investigation of carcinoma metastases.

Considerable difficulties may arise in identifying the Lipiodol histologically, in nodes with very small amounts stored in droplets of about 10 to 15 μ and too small to be identified in the lymphograms. The medium was located in the sinus

mononuclear cells probably reticular cell or macrophages which have phagocytized the grains The grains are homogenous and stain black in specimens prepared with van Gieson Their chemical nature is not clear These observations are in accordance with those of KNUTSON & NORIN (1956)

There seems to be a rough but not clear correlation between estimations of high activity and the amount of demonstrable grains On the other hand occasional nodes of high activity have scant or no visible precipitations possibly due to dissolution during the histotechnical procedure as the substance is water soluble

The rather time consuming method advanced by MELLGREN (1952), which includes vacuum drying and paraffin embedding in vacuo must be used to avoid dissolution and diffusion phenomena these however are of minor importance as stated by MELLGREN et coll (1954) The histologic demonstration of gold pigment in routine preparations may thus be convenient although extremely minute amounts may be overlooked

A reduction of the number of lymphocytes appears to occur in nodes with high activity and areas rich in clusters of ^{198}Au particles even if no traces of lytic cells could be demonstrated (Fig 10) This observation is interesting as a true reduction of cells around the aggregates of ^{198}Au could mean increased cell deaths due to a local radiation effect from the precipitates The local dose of radioactivity in one single lymph node may with the present technique correspond to as much as 1 500 rad per 24 hours and the exposure time multiplies this two or three times As is known from other data, this is sufficient to cause cell depopulation in exposed lymph nodes (TROWELL 1952) an observation that requires investigation One error would be any oedema following irradiation as this would influence to a certain degree the number of cells per unit area No obvious signs of such oedema were seen in the nodes examined The histologic changes observed in connection with the ^{198}Au aggregates were insignificant, and no indications that the existence of the tracer within the node in any way interfered with its capacity to take up the contrast medium were noted This is also borne out by the fact that about 85 per cent of the nodes contained both ^{198}Au and contrast medium

SUMMARY

A method for the evaluation of the distribution of the lymph drainage from different parts of the body to a particular lymph node region is presented It consists of injection of a radioactive tracer in one part of the body and a lymphographic medium in another The method may be of considerable value in clarifying certain unsolved clinical problems associated with surgery and radiotherapy of mammary carcinoma

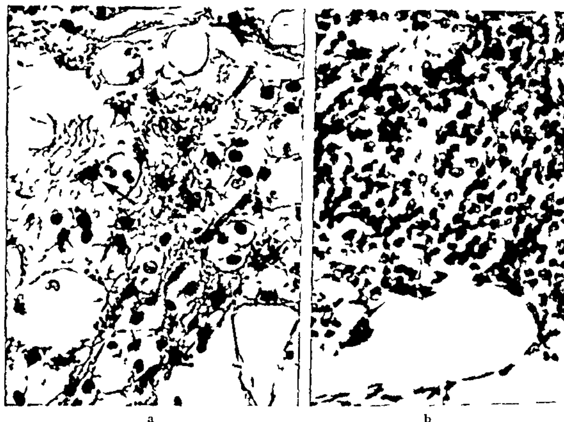


Fig 10 A part of a lymph node with numerous aggregates and grains of gold partly fixed to phagocytizing macrophages (arrow) is shown in (a) which contains few lymphocytes compared with a corresponding area of another lymph node (b) with no radioactivity and no visible gold van Gieson × 530

cells, will surround the droplets (TJERNBERG 1962, AVFRETTE et coll 1964, HULTEN et coll 1966) The existence of such cells around spherical vacuoles clearly differentiated these as Lipiodol residues

The use of a technique that includes fixation and paraffin embedding is necessary for the proper demonstration of carcinoma metastases This puts a limit to the possibilities of staining the Lipiodol, as it is dissolved more or less totally during the procedure Attempts to stain any residues of this contrast medium have so far failed

Demonstration of ^{198}*Au* Clusters of ^{198}Au particles can be demonstrated on routine preparations stained with van Gieson s solution The ^{198}Au forms either single ovoid grains of about 2 μ, or irregular aggregates of 10 to 15 μ They are either diffusely powdered over the specimen or lie in considerable amounts in the sinusoids (Fig 10a), often along the stromal septa They sometimes cover large

FROM THE ONTARIO CANCER TREATMENT AND RESEARCH FOUNDATION OTTAWA CLINIC, OTTAWA, ONTARIO CANADA AND THE DEPARTMENT OF RESEARCH AND ISOTOPES INTERNATIONAL ATOMIC ENERGY AGENCY VIENNA, AUSTRIA

INTERCOMPARISONS OF ABSORBED DOSE IN COBALT 60 TELETHERAPY USING MAILED LiF DOSIMETERS

II Results for Canada and six Asian countries

by

PAUL M PFALZNER and S JAYARAMAN

The object and methods used in the postal dose intercomparisons instituted by the Dosimetry Section of the International Atomic Energy Agency have been described earlier together with results for the first intercomparison which comprised a group of 19 radiotherapy centres in six countries (PFALZNER & MALO ALVAREZ 1968) Since then the same procedures were made available to 14 Canadian participants (Table 1a) and 15 participants in six countries of Asia (Table 1b)

This report gives a brief description of the procedures (essentially those of the first intercomparison) and an analysis of the results for these two groups of participants

Method At approximately monthly intervals each participant received from and returned to IAEA one set of LiF capsules up to a total of three sets A set consisted of three test capsules and two *control* capsules each capsule containing about 150 mg of ConRad type 7 phosphor Participants were instructed to irradiate the test capsules one at a time with a ^{60}Co beam of 10 cm $\times$ 10 cm field size within a plastic water phantom (supplied to them by IAEA) which ensured that the capsule was held at 5 0 cm below the surface of the water The

Presented at the Twelfth International Congress of Radiology Tokyo Japan October 1969 Submitted for publication 2 October 1969

ZUSAMMENFASSUNG

Eine Methode zur Abschätzung des Lymphabflusses von verschiedenen Körperregionen zu bestimmten Lymphdrüsengruppen wird vorgelegt. Sie besteht aus Einspritzung von einem radioaktiven Isotopen in einen Teil des Körpers und einem lymphographischen Mittel in einen anderen Teil. Die Methode ist von besonderem Wert zur Aufklärung klinischer Probleme in der Chirurgie und Strahlentherapie des Brustdrüsenkarzinomes.

RÉSUMÉ

Les auteurs présentent une méthode permettant d'établir la distribution du drainage lymphatique venant des différentes parties du corps et arrivant à un groupe donné de ganglions lymphatiques. Cette méthode consiste à injecter un traceur radioactif dans une partie du corps et à faire une lymphographie dans une autre partie. Elle peut avoir un intérêt considérable pour éclairer certains problèmes cliniques non résolus qui sont en relation avec le traitement chirurgical et radiothérapique du cancer du sein.

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Table 1

Institutes participating in the intercomparison

Table 1a — Canada

Department of Public Health Cancer Clinic	Edmonton Alta
British Columbia Cancer Institute	Vancouver B C
Victoria Cancer Clinic	Victoria B C
Saint John General Hospital	Saint John N B
Victoria General Hospital	Halifax N S
Ontario Cancer Foundation Kingston Clinic	Kingston Ont
Ontario Cancer Foundation London Clinic	London Ont
Ontario Cancer Foundation Thunder Bay Clinic General Hospital	Port Arthur Ont
Ontario Cancer Foundation Windsor Clinic Metropolitan General Hospital	Windsor Ont
Hopital Notre Dame	Montreal Que
Jewish General Hospital	Montreal Que
Queen Elizabeth Hospital	Montreal Que
Allan Blair Memorial Hospital	Regina Sask
University Hospital	Saskatoon Sask

Table 1b — Asia

Government Cancer Institute Maharagama	Ceylon
Tata Memorial Hospital Bombay	India
Cancer Institute Madras	India
National Institute of Radiological Sciences Chiba	Japan
Kyushu University Hospital Fukuoka	Japan
Research Institute for Nuclear Medicine Hiroshima	Japan
Nagoya University Hospital Nagoya	Japan
Radiotherapy Aichi Cancer Centre Nagoya	Japan
Cancer Institute Hospital Tokyo	Japan
Jinnah Post Graduate Medical Centre Karachi	Pakistan
National Taiwan University Hospital Taipei	Taiwan
Provincial Taipei Hospital Taipei	Taiwan
Veterans General Hospital Yangmingshan	Taiwan
Chulalongkorn Hospital and Medical School Bangkok	Thailand
Siriraj Hospital and Medical School Dhonburi	Thailand

absorbed dose delivered to each test capsule was to be 500 rad, the participant following his own method of dosimetry exactly as if treating a patient

Of the two control capsules, one contained unirradiated phosphor, and the other one a sample irradiated to a fixed dose at IAEA prior to being mailed The unirradiated controls served to detect the background and the irradiated

controls to detect any change in stored luminescence due to unknown heating or other effects on the capsules while in transit Further irradiated controls retained at IAEA were used to obtain the luminescence decay curves for the phosphors

The thermoluminescence readings of all capsules were made at the IAEA using a commercial read-out instrument The phosphor from each capsule gave rise to three readings the average of which is the measured value for one capsule Prior to each reading a phosphorescent reference source was inserted into the read out instrument allowing correction to be made for variations in instrumental sensitivity The photomultiplier voltage was kept constant at all times To avoid any error being introduced due to planchet wear and tear no heating planchet was used more than 12 times

At the completion of the series a questionnaire was sent to each participant requesting information on the data used by him in determining the administered dose

Results

Table 2a—2b was prepared on the basis of the information given by the participants in the questionnaire the various factors used by the participants to calculate absorbed dose from a measured exposure are listed The first column of the table lists the institute number which was assigned during the intercomparison to prevent identification by any third parties The other columns show in order the values of the f factor backscatter factor (BSF) central axis percent depth dose (% DD) and source to surface distance (SSD) tissue air ratio (TAR) and displacement factor (C_D) The information given by the participants indicated that four methods of deriving the absorbed dose from the exposure were in use The formulas are shown in Table 4

In order to separate the effects of discrepancies in exposure determination from those due to the factors used in calculation of absorbed dose uniform values for the various multiplicative terms were adopted as shown in Table 5 It should be noted that while a value of 0 985 was taken for the displacement factor in all cases the value of this factor is not well known for instruments other than the Farmer Baldwin thimble ionization chamber In several cases the kind of measuring chamber used was not reported (institutes 81 85 42 and 57) In the last column of Table 2a—2b the normalizing factors are given which represent the correction to the results due to adoption of the uniform values in Table 5

Table 3a—3b shows the results of the read out evaluations for the test capsules irradiated — as determined by the participants — to an absorbed dose of 500 rad Since the thermoluminescence readings were taken at any time between 15 and 160 days after irradiation the results have been normalized to 50 days

Table 2

Factors used by participants to calculate absorbed dose

Table 2a — Canada

Institute No	f rad/R	Field size 10 cm × 10 cm			C_D	Normalizing factors
		BSF	At 5 cm depth			
			% DD_{SSD}	TAR		
71	0 965	—	—	0 888	1 0	0 996
72	0 97	1 026	$78\ 2_{10}$	—	1 0	1 008
73	1 0	—	—	0 893	1 0	1 038
74	0 957	—	—	0 893	1 0	0 993
75	0 965	1 025	$78\ 5_{10}$	—	1 0	1 005
76	0 965	—	—	—	1 0	1 015
77	0 957	1 036	$78\ 5_{10}$	—	0 985 and 0 98	0 990
78	0 965	—	—	0 893	1 0	1 00[illegible]
79	0 957	—	—	0 895	0 985	0 981
80	0 974	1 026	$75\ 9_{10}$	—	1 0	1 016
81	0 96	—	—	—	1 0	1 010
83	0 957	1 036	$75\ 9_{10}$	—	0 985	0 993
85	0 965	—	—	—	1 0	1 015
86	0 97	1 029	$78\ 2_{10}$	—	0 986	0 996

Table 2b — Asia

Institute No	f rad/R	BSF	% DD_{SSD}	TAR	C_D	Normalizing factors
42	Details not available					
43	0 973	1 026	$74\ 0_{10}$	—	1 0	1 024 and 1 003*
44	1 000	—	$74\ 0_{10}$	—	1 0	1 073*
45	0 964	—	—	0 893	1 0	1 001
46	0 975	1 026	$75\ 9_{10}$	—	1 0	1 031 and 1 039*
47	0 974	1 026	$78\ 0_{10}$	—	1 0	1 038*
48	0 960	1 135	$74\ 0_{5}$	—	1 0	1 015
49	0 965	1 071	76 0	—	1 0	0 983
50	0 944***	—	—	0 890	—	0 977
51	0 974	—	—	0 890	1 0	0 993
53	0 974	—	—	0 893	1 0	1 011
55	0 960	—	—	0 893	1 0	0 997**
56	0 974	1 026	$75\ 0_{15}$	—	1 0	1 034*
57	Details not available					
58	0 965	—	—	0 905	1 0	1 015

* Includes inverse square law correction ** A cobalt 60 decay correction was also applied

*** Represents C_λ

post irradiation using phosphor decay corrections obtained from the irradiated control capsules The unirradiated control capsules in all cases gave a reading of less than 1 % of the irradiated test capsules A standard deviation of 2 6 % was found for the irradiated controls

In Table 3a—3b the first column lists the institute number next are shown the percentage differences between the mean of all capsule readings for each institute and the overall mean together with the tandard deviation of the in stitute s mean The institutional means were recalculated (using the normalizing factors of Table 2a—2b) and the percentage differences between the institute s normalized mean and the new overall mean are given in column 4 of Table 3a—3b Finally the last two columns list the make of measuring instrument used by the participant, and the institute s method of absorbed dose calculation ac cording to the formulas shown in Table 4

Canada Considering first the factors used by participants in their calculations (Table 2a) it will be noted that there exists some disagreement concerning the f factor the two values 0 965 and 0 957 occur most frequently representing the roentgen to-rad conversion factor as obtained from ICRU Report 10b (ref 8) for a photon energy of 1 25 MeV for water and muscle respectively, in earlier ICRU reports these values were given as 0 974 and 0 965, respectively, and hence it is not certain whether the five institutes using 0 965 intended this to be the value for water or for muscle The value 0 97 was used by two participants probably representing the earlier ICRU f values 0 96 occurs once and so does 0 974 the earlier value for water One participant equated roentgen and rad The backscatter factor for a 10 cm × 10 cm cobalt field was taken as 1 026 or 1 025 by three participants corresponding to the value given in Brit J Radiol Supplement 10 (ref 4) Two participants adopted the higher value of 1 036 and a value of 1 029 was used in one instance The per cent depth dose values of 78 5 % at 80 cm SSD and 75 9 % at 60 cm SSD, each used by two partic ipants correspond to the values given in Supplement 10 78 2 % at 80 cm SSD was used by two others The most commonly used value for the tissue air ratio was 0 893 (ref 4) an earlier value of 0 888 and the value 0 895 occurred in single instances A displacement factor C_D was used by four participants of these only two (institutes 77 and 83) made use of the backscatter factor of 1 036 (ref 3) which requires the use of the factor C_D all other participants used the older lightly lower values of backscatter factor or of tissue air ratio and (except for institutes 79 and 86) ignored the displacement factor a procedure which is justifiable inasmuch as the *effects* tend to cancel

The last column of Table 2a gives the normalizing factors which reflect the disagreement between institutes in their use of the factors involved in calculation

Table 3

Results of intercomparison of cobalt 60 absorbed dose

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
Table 3a — Canadian participants					
71	2 2	5 3	1 4	N R C	3
72	— 0 1	2 4	0 3	N R C	4
73	— 1 8	3 2	1 5	Victoreen	3
74	4 8	4 3	3 7	Farmer S S	3
75	— 3 5	3 8	— 3 4	Victoreen	4
76	— 6 8	3 9	— 5 8	Victoreen	1
77	— 2 7	2 6	— 4 1	Farmer S S	4 and 1
78	2 7	4 3	2 5	Victoreen	3
79	3 1	2 8	0 7	Farmer S S	3
80	— 1 2	5 0	0 0	N R C	4
81	3 1	3 0	3 7	Ionization Chamber	1
83	2 5	2 3	1 4	Farmer S S	4
85	— 3 1	2 7	— 2 1	?	1
86	1 1	2 9	0 3	N R C	4

Standard deviation 3 3 % of mean 2 9 % of mean

Extreme difference 11 6 % of mean 9 5 % of mean

The two means differ by 0 4 %

* Not recalculated ** Baldwin Ionex = Baldwin Ionex ionization meter MK 3 Baldwin Reference = Baldwin Farmer electrometer with reference chamber Farmer S S = Farmer Baldwin secondary standard dosemeter N R C = National Research Council of Canada reference instrument (aluminium cavity ionization chamber with Townsend balance electrometer) Radocon = Victoreen dosemeter Siemens = Siemens dosemeter Victoreen = Victoreen condenser R meter (25 R thimble chamber)

of absorbed dose from a measured exposure It is seen that these are quite small, generally less than 1 %, with a maximum of 3 8 % for the participant using an f factor of 1 0

Turning next to Table 3a, it is seen that the agreement between institutes with respect to absorbed dose (column 2) is better than 5 % for all except one participant, with maximum deviations from the mean of + 4 8 % and — 6 8 %, the individual standard deviations also being 5 % or less with one exception Although the normalizing factors, as mentioned are generally small, it is seen

Table 3 (co t)

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
Table 3b — As an participants					
42	—16 3	4 3	—17 3	?	?
43	— 8 6	3 1	— 8 7	Farmer S S	1 and 4
44	— 5 0	10 8	0 5	Victoreen	2
45	— 6 1	7 9	— 7 0	Farmer S S	3
46	7 4	3 5	9 7	Baldwin Ionex	1 and 4
47	5 3	2 5	8 1	Victoreen	4
48	— 1 1	4 4	— 0 7	Baldwin Ionex	4
49	6 6	3 9	3 7	Victoreen	4
50	3 3	3 9	— 0 9	Farmer S S	3
51	6 4	5 7	4 6	Fricke and Radocon	3
53	7 6	1 5	7 5	Baldwin Reference	3
55	15 4	2 0	13 8	Siemens	3
56	—10 8	9 1	— 8 9	Siemens	4
57	9 9	3 8	1 8	?	?
58	— 7 3	4 0	— 7 0	Farmer S S	3

Standard deviation 8 7 of mean — 8 4 of mean
Extreme difference 31 7 of mean — 31 1 % of mean
The two means differ by 1 1

(column 4) that the percent deviations from the normalized mean are reduced for eleven out of fourteen participants the maximum deviations from the mean changing to + 3 7 % and — 5 8 % with a corresponding small decrease in the standard deviation expressed as a percentage of the mean The differences among institutes shown in column 4 now correspond chiefly to differences in exposure determination and are seen to be less than 4 % in the great majority of cases

Participants used three kinds of measuring instruments with numbers equally divided between the Farmer and N R C secondary standard and the Victoreen R meter It is noteworthy that the four institutes employing the reference instrument provided by the National Research Council of Canada especially for cobalt 60 beam calibrations (ref 1) had some of the smallest per cent deviations For two institutes details of the instruments used were not available

Table 3a further shows that three methods of dose calculation were used by the participants Methods 3 and 4 both involving exposure measurements in air

Table 3

Results of intercomparison of cobalt 60 absorbed dose

Institute No.	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No.
Table 3a — Canadian participants					
71	2.2	5.3	1.4	N.R.C.	3
72	—0.1	2.4	0.3	N.R.C.	4
73	—1.8	3.2	1.5	Victoreen	3
74	4.8	4.3	3.7	Farmer S.S.	3
75	—3.5	3.8	—3.4	Victoreen	4
76	—6.8	3.9	—5.8	Victoreen	1
77	—2.7	2.6	—4.1	Farmer S.S.	4 and 1
78	2.7	4.3	2.5	Victoreen	3
79	3.1	2.8	0.7	Farmer S.S.	3
80	—1.2	5.0	0.0	N.R.C.	4
81	3.1	3.0	3.7	Ionization Chamber	1
83	2.5	2.3	1.4	Farmer S.S.	4
85	—3.1	2.7	—2.1	?	1
86	1.1	2.9	0.3	N.R.C.	4
Standard deviation	3.3 % of mean		2.9 % of mean		
Extreme difference	11.6 % of mean		9.5 % of mean		

The two means differ by 0.4 %

* Not recalculated ** Baldwin Ionex = Baldwin Ionex ionization meter MK 3. Baldwin Reference = Baldwin Farmer electrometer with reference chamber. Farmer S.S. = Farmer Baldwin secondary standard dosemeter. N.R.C. = National Research Council of Canada reference instrument (aluminium cavity ionization chamber with Townsend balance electrometer). Radocon = Victoreen dosemeter. Siemens = Siemens dosemeter. Victoreen = Victoreen condenser R meter (25 R thimble chamber).

of absorbed dose from a measured exposure. It is seen that these are quite small, generally less than 1 %, with a maximum of 3.8 % for the participant using an f factor of 1.0.

Turning next to Table 3a, it is seen that the agreement between institutes with respect to absorbed dose (column 2) is better than 5 % for all except one participant, with maximum deviations from the mean of +4.8 % and —6.8 %, the individual standard deviations also being 5 % or less with one exception. Although the normalizing factors, as mentioned, are generally small, it is seen

Table 3 (*cont*)

Institute No	Per cent deviation from overall mean	Standard deviation per cent	Per cent deviation from normalized mean	Measuring chamber**	Method No
Table 3b — Asian participants					
42	—16 3	4 3	—17 3*	?	?
43	— 8 6	3 1	— 8 7	Farmer S S	1 and 4
44	— 5 0	10 8	0 5	Victoreen	2
45	— 6 1	7 9	— 7 0	Farmer S S	3
46	7 4	3 5	9 7	Baldwin Ionex	1 and 4
47	5 3	2 5	8 1	Victoreen	4
48	— 1 1	4 4	— 0 7	Baldwin Ionex	4
49	6 6	3 9	3 7	Victoreen	4
50	3 3	3 9	— 0 2	Farmer S S	3
51	6 4	5 7	4 6	Fricke and Radocon	3
53	7 6	1 5	7	Baldwin Reference	3
55	15 4	2 0	13 8	Siemens	3
56	—10 8	9 1	— 8 9	Siemens	4
57	2 9	3 8	1 8	?	?
58	— 7 3	4 0	— 7 0	Farmer S S	3
Standard deviation	8 7 of mean		8 4 of mean		
Extreme difference	31 7 of mean		31 1 of mean		

The two means differ by 1 1

(column 4) that the percent deviations from the normalized mean are reduced for eleven out of fourteen participants the maximum deviations from the mean changing to + 3 7 % and — 5 8 % with a corresponding small decrease in the standard deviation expressed as a percentage of the mean The differences among institutes shown in column 4 now correspond chiefly to differences in exposure determination and are seen to be less than 4 % in the great majority of cases

Participants used three kinds of measuring instruments with numbers equally divided between the Farmer and N R C secondary standard and the Victoreen R meter It is noteworthy that the four institutes employing the reference instrument provided by the National Research Council of Canada especially for cobalt 60 beam calibrations (ref 1) had some of the smallest per cent deviations For two institutes details of the instruments used were not available

Table 3a further shows that three methods of dose calculation were used by the participants Methods 3 and 4 both involving exposure measurements in air

Table 4

Formulas for calculation of absorbed dose from measured exposure by different methods

Method		
1		$D_5 = f \cdot C_D \cdot {}_W X_5$
2		$D_5 = f \cdot C_D \cdot {}_W X_{0.5} \cdot P_5$
3		$D_5 = f \cdot C_D \cdot {}_A X_5 \cdot T_5$
4		$D_5 = f \cdot C_D \cdot {}_A X_{0.5} \cdot P_5 \cdot B$
	D_5	Absorbed dose in water at (SSD + 5) cm, i.e. at position of LiF capsule
	${}_W X_5$	Exposure measured in water at (SSD + 5) cm
	${}_W X_{0.5}$	Exposure measured in water at (SSD + 0.5) cm
	${}_A X_5$	Exposure measured in air at (SSD + 5) cm
	${}_A X_{0.5}$	Exposure measured in air at (SSD + 0.5) cm
	f	Rad per roentgen factor (rad/R)
	C_D	Displacement factor
	P_5	Central axis fractional depth dose at 5 cm depth
	T_5	Tissue air ratio at 5 cm depth
	B	Backscatter factor
	SSD	Source-to-surface distance (cm)

were followed by a majority, whereas method 1, based on measurements in water, (as recommended by ICRU and the 'H.P.A. Code of Practice'), was used by three participants only (76, 81 and 85). This method, for purposes of this intercomparison, does not involve backscatter, per cent depth dose or tissue air ratio, requiring two factors only (in addition to the chamber quality factor and — for unsealed ionization chambers — the temperature pressure factor). The three institutes employing this method did not make use of a displacement factor, however. Method 4, although calling for the largest number of multiplicative terms, was used by six institutes. One participant used methods 4 and 1 as checks against one another.

4.sia Table 2b shows that various f factors are in use, the value preferred by six participants clustering around 0.974, which is the older value for water; three participants used 0.965 or 0.964, one chose 0.96, and an unusally low value of 0.944 (this is probably the C_λ factor, which includes f and C_D, to be used with method No. 1 as recommended in 1964 in the H.P.A. Code of Practice for dosemeters calibrated at an X-ray energy of 2 MV) occurs in one instance. One participant did not report a value for this factor, and it is assumed that conversion to rad was not carried out. Details were not available for two institutes. The backscatter factor given in the depth dose tables (ref. 4) was used in most cases, with one participant (48) employing the higher value of 1.035. The per cent depth doses quoted are for shorter distances than in Canada, and while values for 50 and 60 cm SSD agree with those given in

Table 5

Values adopted for purposes of normalization — Factors applying to cobalt 60 10 cm × 10 cm field 5 cm depth — Values of P_5 for 55 cm and 70 cm SSD were calculated according to eq (13) in reference (6)*

			SSD cm	P (ref 4)
f (water) rad/R	0 965	(ref 8)	50	0 740
C_D	0 985	(ref 4)	55	0 750*
T	0 905	(ref 5)	60	0 759
B	1 035	(ref 5)	70	0 774*
Half life	5 26 y	(ref 9)	80	0 785

ref 4 the values at 70 cm SSD are discrepant The tissue air ratios again correspond closely to those given in ref 4 with one exception (58) where the higher value of 0 905 (ref 5) was used It must be noted that the two institutes (48 and 58) using the higher values of backscatter and of tissue air ratio failed as was the case in Canada to make use of a displacement factor One participant (51) appears to have obviated the use of this factor by basing his calibration on measurements with a Fricke dosemeter

The majority of Asian participants used the older slightly lower values of the factors and none of them applied a displacement factor The normalizing factors tend to be larger for these participants and in five instances (43 44 46 47 and 56) include small corrections for inverse square law terms omitted by the participant In one instance (55) radioactive decay calculations appeared faulty and were corrected for purposes of normalization by an additional factor not included in the listed normalizing factors

Considering next Table 3b it is seen that disagreement between institutes with respect to absorbed dose (column 2) reaches a maximum of about 32 % (+ 15 4 % and — 16 3 %, respectively) with a standard deviation of about 9 % Large individual deviations from the mean are found for three institutes in particular Only four participants have deviations from the mean of 5 % or less as compared to only one having a deviation of more than 5 % for Canada As a result of using the normalizing factors some reductions do occur in the per cent deviations in nine instances (column 4) but in the remaining six the results were less satisfactory

A greater variety of measuring instruments were used by the Asian participants with the Farmer secondary standard being slightly in the lead over the Victoreen chamber followed by Baldwin Ionex Siemens dosemeter Baldwin reference condenser chamber and Victoreen Radocon used in combination with a Fricke dosemeter

Table 4

Formulas for calculation of absorbed dose from measured exposure by different methods

Method	
1	$D_5 = f\ C_D\ {}_{W}X_{5}$
2	$D_5 = f\ C_D\ {}_{W}X_{0.5}\ P_5$
3	$D_5 = f\ C_D\ {}_{A}X_{5}\ T_5$
4	$D_5 = f\ C_D\ {}_{A}X_{0.5}\ P_5\ B$

D_5	Absorbed dose in water at (SSD + 5) cm i e at position of LiF capsule
${}_{W}X_{5}$	Exposure measured in water at (SSD + 5) cm
${}_{W}X_{0.5}$	Exposure measured in water at (SSD + 0 5) cm
${}_{A}X_{5}$	Exposure measured in air at (SSD + 5) cm
${}_{A}X_{0.5}$	Exposure measured in air at (SSD + 0 5) cm
f	Rad per roentgen factor (rad/R)
C_D	Displacement factor
P_5	Central axis fractional depth dose at 5 cm depth
T_5	*Tissue air ratio at 5 cm depth*
B	Backscatter factor
SSD	Source to surface distance (cm)

were followed by a majority, whereas method 1, based on measurements in water, (as recommended by ICRU and the 'H P A Code of Practice'), was used by three participants only (76, 81 and 85) This method, for purposes of this intercomparison, does not involve backscatter, per cent depth dose or tissue air ratio, requiring two factors only (in addition to the chamber quality factor and — for unsealed ionization chambers — the temperature pressure factor) The three institutes employing this method did not make use of a displacement factor, however Method 4, although calling for the largest number of multiplicative terms, was used by six institutes One participant used methods 4 and 1 as checks against one another

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Table 5

Values adopted for purposes of normalization — Factors applying to cobalt 60 10 cm × 10 cm field 5 cm depth— Values of P_{δ} for 55 cm and 70 cm SSD were calculated according to eq (13) in reference (6)*

			SSD cm	P (ref 4)
f (water) rad/R	0 965	(ref 8)	50	0 740
C_D	0 985	(ref 4)	55	0 750*
T	0 905	(ref 5)	60	0 759
B	1 035	(ref 5)	70	0 774*
Half life	5 26 y	(ref 9)	80	0 785

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The majority of Asian participants used the older slightly lower values of the factors and none of them applied a displacement factor The normalizing factors tend to be larger for these participants and in five instances (43 44 46 47 and 56) include small corrections for inverse square law terms omitted by the participant In one instance (55), radioactive decay calculations appeared faulty and were corrected for purposes of normalization by an additional factor not included in the listed normalizing factors

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Method	
1	$D_5 = f \; C_D \; {}_W X_5$
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3	$D_5 = f \; C_D \; {}_A X_5 \; T_5$
4	$D_5 = f \; C_D \; {}_A X_{0.5} \; P_5 \; B$

D_5	Absorbed dose in water at (SSD + 5) cm i.e. at position of LiF capsule
${}_W X_5$	Exposure measured in water at (SSD + 5) cm
${}_W X_{0.5}$	Exposure measured in water at (SSD + 0.5) cm
${}_A X_5$	Exposure measured in air at (SSD + 5) cm
${}_A X_{0.5}$	Exposure measured in air at (SSD + 0.5) cm
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P_5	Central axis fractional depth dose at 5 cm depth
T_5	Tissue air ratio at 5 cm depth
B	Backscatter factor
SSD	Source to surface distance (cm)

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Asia. Table 2b shows that various f factors are in use, the value preferred by six participants clustering around 0.974, which is the older value for water, three participants used 0.965 or 0.964, one chose 0.96, and an unusually low value of 0.944 (this is probably the C_λ factor, which includes f and C_D, to be used with method No. 1 as recommended in 1964 in the H.P.A. Code of Practice for dosemeters calibrated at an X ray energy of 2 MV) occurs in one instance. One participant did not report a value for this factor, and it is assumed that conversion to rad was not carried out. Details were not available for two institutes. The backscatter factor given in the depth dose tables (ref. 4) was used in most cases, with one participant (48) employing the higher value of 1.035. The per cent depth doses quoted are for shorter distances than in Canada, and while values for 50 and 60 cm SSD agree with those given in

Table 5

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			SSD cm	P (ref 4)
f (water) rad/R	0 965	(ref 8)	50	0 740
C_D	0 985	(ref 4)	55	0 7 0*
T	0 905	(ref 5)	60	0 759
B	1 035	(ref 5)	70	0 774*
Half lif	5 26 y	(ref 9)	80	0 785

ref 4 the values at 70 cm SSD are discrepant The tissue air ratios again correspond closely to those given in ref 4, with one exception (58) where the higher value of 0 905 (ref 5) was used It must be noted that the two institutes (48 and 58) using the higher values of backscatter and of tissue air ratio failed as was the case in Canada to make use of a displacement factor One participant (51) appears to have obviated the use of this factor by basing his calibration on measurements with a Fricke dosemeter

The majority of Asian participants used the older, slightly lower values of the factors and none of them applied a displacement factor The normalizing factors tend to be larger for these participants and in five instances (43 44 46 47 and 56) include small corrections for inverse square law terms omitted by the participant In one instance (55) radioactive decay calculations appeared faulty and were corrected for purposes of normalization by an additional factor not included in the listed normalizing factors

Considering next Table 3b it is seen that disagreement between institutes with respect to absorbed dose (column 2) reaches a maximum of about 32 % (+ 15 4 % and — 16 3 % respectively) with a standard deviation of about 9 % Large individual deviations from the mean are found for three institutes in particular Only four participants have deviations from the mean of 5 % or less as compared to only one having a deviation of more than 5 % for Canada As a result of using the normalizing factors some reductions do occur in the per cent deviations in nine instances (column 4) but in the remaining six the results were less satisfactory

A greater variety of measuring instruments were used by the Asian participants with the Farmer secondary standard being slightly in the lead over the Victoreen chamber followed by Baldwin Ionex Siemens dosemeter Baldwin reference condenser chamber and Victoreen Radocon used in combination with a Fricke dosemeter

Finally it is seen that most participants also preferred methods involving in air exposure measurements (methods 3 and 4) Method 1 was used in two instances only, both times in combination with method 4, and method 2 was used in a single instance

Discussion

The results for Canada are similar to those of the first IAEA postal dose intercomparison (ref 7) which showed extreme differences between institutes of 14 3 % and 9 3 % with respect to absorbed dose and normalized dose, respectively The present results give a value of 11 6 % for the extreme difference between institutes with respect to absorbed dose, and a corresponding figure of 9 5 % with respect to the normalized values This residual difference of about 10 % between institutes appears to be larger than is desirable, but is, of course, considerably better than the results found for the Asian participants where the differences reach up to 30 % Some of the Asian institutes are certainly doing careful work in every way comparable to that done in other parts of the world but other, perhaps more isolated clinics, have greater difficulties in following modern procedures and maintaining good calibrations

It is clear, in any case, that only a small part of the differences between clinics is due to the factors used to convert exposure to absorbed dose Thus a basic improvement is only likely to result from (1) maintaining properly calibrated, reliable dosemeters in each clinic, (2) adopting a reproducible dosimetric procedure (e g using a standard calibration phantom), and (3) using accepted factors to calculate absorbed dose from exposure It should be noted that if method 1 is adopted this requires agreement on two factors only (f and C_D), and the user may select whatever per cent depth dose values (or tissue air ratios) he feels are most appropriate for his conditions to calculate doses at any other points

It appears that the procedure — equivalent to method 1 — recommended by ICRU (ref 2) for calibration is not yet used to any large extent, nor is the need for a displacement factor well understood In methods 3 and 4, which are at present widely used, the displacement factor is necessary as a consequence of the definition of tissue air ratio (and, by implication, of backscatter factor) in terms of a ratio of two absorbed doses

It should be noted that the factor C_D, which occurs in all four methods listed in Table 4, has two different derivations depending on whether (1) an exposure is measured inside a medium such as water (methods 1 and 2), or (2) an exposure is measured in air and then converted to absorbed dose in water or soft tissue (as in methods 3 and 4) For case (1) only, the correction factor C_D will vary with the dimensions and construction of the measuring chamber, being

due to an actual displacement of medium owing to the introduction of the chamber for case (2) the factor C_D is not a function of the physical presence of a measuring chamber but arises from the concept of a volume of phantom material just large enough to provide the maximum electronic build up at the point of measurement (definition of tissue air ratio, ref 2 page 5), and is thus a function of the radiation energy only It is fortunate that for chambers such as the Farmer Baldwin thimble chamber, at any rate, the values of the two factors seem to be nearly identical

Finally in conclusion it may be stated that the postal intercomparison of absorbed dose using LiF dosimeters as instituted by the IAEA has proven to be practical and effective in helping to evaluate and improve accuracy of dose delivery in teletherapy During the past year this technique has been further standardized and refined and is being made available by IAEA to an increasing number of radiotherapy centres There is every indication that this IAEA service is having a significant effect towards achieving international consistency and comparability of radiation dosage in the treatment of cancer

Acknowledgements

The authors are indebted to Dr W H Henry (N R C Ottawa) and Dr R Loevinger (NBS Washington) for helpful comments and are pleased to acknowledge the valuable assistance of S Malo Alvarez and S Tsialas in the preparation and evaluation of phosphor samples The work described here was carried out while the senior author (P M P) was Head of the Dosimetry Section International Atomic Energy Agency Vienna Austria on leave of absence granted by the Ontario Cancer Treatment and Research Foundation Toronto Canada The views expressed are those of the authors and not necessarily those of the International Atomic Energy Agency

SUMMARY

A series of dosimeter capsules containing LiF phosphor were distributed by the International Atomic Energy Agency to 14 Canadian radiotherapy centres and to 15 centres in six countries in Asia The dosimeters were irradiated with a cobalt 60 beam at a depth of 5 cm in water to an absorbed dose calculated by the participants to be 500 rad and returned to IAEA by *mail for read-out* The maximum difference in absorbed dose between participating centres was found to be 11 6 % for Canada and 31 7 % for the Asian countries with per cent standard deviations of 3 3 % and 8 7 % for the respective overall mean doses The lack of uniformity in the dosimetric methods and in the factors used by participants to convert exposure to absorbed dose indicates a need for closer international collaboration and standardization in medical radiation dosimetry

ZUSAMMENFASSUNG

Eine Reihe von mit LiF Phosphor gefullten Dosimeter kapseln wurde von der Internationalen Atomenergiebehorde (IAEO) an 14 Radiotherapie Zentren in Kanada sowie an

15 Zentren in sechs asiatischen Lindern versandt Den Dosimetern wurde in einer Tiefe von 5 cm Wasser eine von den Teilnehmern berechnete Energiedosis von 500 rad Kobalt strahlung verabreicht Zur Auswertung wurden die Dosimeter per Post an die IAEO re tourniert Es ergab sich eine zwischen verschiedenen Teilnehmern bestehende Maximal differenz in Energiedosis von 11 6 % in Kanada, bzw 31 7 % in Asien mit einer prozentualen Standardabweichung fur die Durchschnittswerte von 3 3 %, bzw 8 7 % Die ver schiedenen von den Teilnehmern benutzten dosimetrischen Methoden sowie Faktoren zur Umrechnung von Bestrahlungs auf Energiedosis weisen auf die Notwendigkeit hin engere internationale Zusammenarbeit und Standardisierung in der medizinischen Strahlendosi metrie zu fordern

RÉSUMÉ

Une serie de capsules dosimetriques contenant des scintillateurs de LiF ont ete distribuees par l Agence Internationale de l Energie Atomique a 14 centres de radiotherapie canadiens et a 15 centres situes dans 6 pays asiatiques Les dosimetres ont eté irradies par le rayonnement du cobalt 60 a une profondeur de 5 cm dans l eau jusqu a une dose absorbee calculee *par les participants comme etant égale à 500 rad et les dosimetres ont ete retournes a l AIEA* par la poste pour lecture de la dose Le maximum de difference de doses absorbees entre les differents centres participant a l experience a éte de 11 6 % pour le Canada et de 31,7 % pour les pays asiatiques avec des déviations standard de 3 3 % et de 8 7 % pour les doses moyennes respectives *Le manque d uniformite dans les methodes dosimetriques et dans les* facteurs utilises par les participants pour convertir la dose d exposition en dose absorbee indique la nécessite d une collaboration internationale plus etroite et d une standardisation dans la dosimetrie des radiations en medecine

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FROM THE DEPARTMENT OF RADIOTHERAPY, LAC/USC MEDICAL CENTER LOS ANGELES, CALIFORNIA U S A

OBJECTIVE PROCEDURE OF CONSTRUCTING STAGES IN CANCER

Its application in recurrent carcinoma of the corpus uteri

by

SAMUEL S KUROHARA and FREDERICK W GEORGE III

Staging has been an empirically developed procedure by which clinical oncologists have tried to classify a cancer entity into levels of advancement these may then be used as a means of predicting the behaviour of cancer in an individual or in groups of patients The clinical variables most commonly employed in the development of such classifications have been the anatomic sites and the extent of involvement Less often used are certain other of the characteristics of the disease

A functionally satisfactory system of clinical cancer staging should (1) separate patients with varyingly advanced lesions into distinct groups each group possessing (a) a consistently predictable and different level of prognosis and constituting (b) a roughly equal percentage of the total patient population (2) be simple enough to be widely applicable (3) provide an effective and reliable means of comparing different methods of treatment and (4) consistently predict the outcome of the disease in individual patients taking into account alterations induced by additional variables such as methods of treatment

Submitted for publication 21 August 1969

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part of a more extensive study on clinical features and prognoses in 300 cases of recurrent carcinoma of the corpus uteri (KUROHARA et coll 1969) treated at the Roswell Park Memorial Institute

Measurements Assessment of the clinical stage of the malignant process in this group of patients and in any group so utilized should be performed by methods generally applicable to all patients in the sample population These ordinarily include the recording of the medical history, and the physical examination and laboratory tests at the time of admission Biopsy of the lesion complete blood count urinalysis fasting blood sugar blood urea nitrogen veneral disease and liver function tests and chest and metastatic survey roentgenograms will probably be the rule in all cases considered to have cancer Certain special procedures of particular importance in the specific type of cancer being considered are almost always employed in most medical centers. For example, a pelvic examination under anesthesia with a diagnostic curettage of the uterine cavity is almost invariably carried out in cases of gynecologic cancer while urography is usual in cancer of the cervix or testis and paranasal sinus roentgenograms in cases of carcinoma of the antrum

In the present study assessment of the extent of involvement of the anatomic sites was obtained by the above mentioned routine examinations This had been carefully recorded in the medical records of the cases either by word or by diagram biospy specimens and roentgenograms were reviewed

The anatomic sites of potential involvement by recurrent cancer were defined as nearly as the available data permitted Pelvic organs or regions directly visible or palpable were subdivided into segments For example the vagina was subdivided into upper and lower halves (introitus and minor lip being included in the latter) The upper part of the vagina was subdivided into anterior posterior right lateral and left lateral walls and the apex or cuff The lower part of the vagina was similarly subdivided excepting the last mentioned subdivision The true pelvis was subdivided into regions surrounding the vagina on the right and left aspects as well as left superiorly and into regions close to the pelvic wall bilaterally Lesions involving the latter sites were considered fixed to the pelvic wall

Distant sites of spread were less discretely defined than the local or regional sites For example abdominal involvement was designated by the findings of a mass in the right upper quadrant midabdomen or elsewhere or of multiple masses or ascites Thoracic sites were designated as lung pleura and mediastinum skeletal sites as pelvis spine ribs

Each of the anatomic sites were identified numerically These numerical scores were punched on IBM cards for computer purposes Zero scores were reserved

The definitions and other criteria that have established the advancement levels and related guidelines in most existing staging systems have to date been derived by more or less a trial and error method In a somewhat arbitrary manner, individuals or committees have proposed, considered and perhaps adopted certain patterns of anatomic spread, or extent of involvement, as hopefully valid indices by which to predict the outcome and thus establish the prognosis These empirically constructed staging systems have been produced in an almost endless number of variations for virtually every type of cancer However, on careful evaluation only a handfull of them can be shown to meet the criteria for a satisfactorily functioning clinical cancer staging system

The major cause of the general ineffectiveness of cancer staging systems has been the lack of careful analysis, prior to the development of the system, to identify the clinical factors essential to a proper staging Such identified factors should always correlate with the outcome of the disease in similar patient populations An arbitrary selection of these important components cannot ensure categorization of patients into well defined groups If the prognostic distinctions of each of the component clinical factors are not identified before combining them into the various levels of stage a mixing of factors with different degrees of prognostic distinctions within the same stage will inevitably occur Each such mixing may conceal or distort important patient information and play a role in concentrating disproportionate numbers of patients to one or two levels of staging, thus producing an imbalanced system

The following method is advanced as a logical and objective approach to the problem of constructing staging systems and may be summarized as follows

Variables (or clinical patient factors) are provisionally selected as representing (1) valid measures of prognosis in a specific variety of cancer, and (2) findings readily obtainable under ordinary clinical conditions, decomposed as discretely as possible into their essential ingredients

Survival rates of each separate subset of patients manifesting all the variables, selected either alone or in combinations, are computed from substantial clinical experience by means of appropriate statistical techniques

Subsets with one or more factors having similar survival characteristics are then combined into a few levels or categories each involving approximately equal numbers of the patient population analyzed and each association with distinctly prognostic features

Material and Methods

The construction of a cancer staging system is illustrated by a series of 149 cases of endometrial carcinoma recurrent after total hysterectomy The material is

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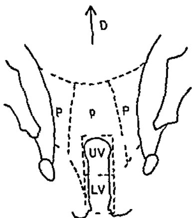


Fig 2 Anatomic sites of involvement UV—upper vagina LV—lower vagina p—pelvis (not fixed to pelvic wall) P—pelvis (fixed to pelvic wall) D—distant

statistical computation could have been used a special computer program (BASCO & KUROHARA in preparation) designed to generate a matrix of simple or complex or both variables and to perform analysis was employed in order to save time and labor and lessen the chances of human error common in manual methods

A schematic diagram is shown in Fig 1 for the matrix of bivariate distribution of scores produced by the program Variables having any of three kinds of measured scores can be handled in the columns and rows Those having *continuous* scores (e g age uterine cavity depth survival time) those having *ordinal* scores (e g histologic grade clinical stage gravida), and those having *nominal* scores (e g sex color histology treatment categories) can be tabulated against each other like against like or like against unlike score type variables

The superscript a is used to indicate that partitioning of original scores of one or more variables can be degenerated into ordered or non ordered scores with uniform or non uniform intervals of the original These can be combined with ordinal or nominal or both scores of other kinds of variables to produce a new complex nominal variable in the column or rows or both For example, the first column may be a symptom complex group into which are incorporated all patients 25 to 45 years of age with 2 to 7 months symptom duration and with a hemoglobin of 8 to 10 mg % the econd column a symptom complex group including those of 60 to 65 years with 0 to 2 months symptom duration with a hemoglobin of 10 to 15 mg % and with no previous history of surgery The various levels of histologic grade of cancer or levels or classes of some other complex variable may be assigned in the rows The relative fequency of distribution for the entire table for column or rows or both can be obtained by option

CROSS TABULATION

COLUMN

CONTINUOUS ORDINAL NOMINAL	SCALE → ORDER 1	2	3	4
	CLASS I $V + V_2 \; V_3^1 + V_4^1$	II $V^2 + V_2^2 + V_3^3 + V_4^2$	III $V^3 + V_5 \; V_6 + V_4^3$	IV $V^4 \; V_5 + V_6^3 + V_5^2 \; V$
ROW SCALE 1 CLASS I $V_8 \; V_9 + V_7^2$				
2 II $V^2 + V_9^3 + V_{10}$				
3 III $V_3^2 \; V_3 + V_6^2 + V_4^6$				
4 IV $V_{30} + V_{30}^2 + V_{10}^4$				

GENERAL SELECTOR VARIABLES

$V_5^1 \; V_5^2 \; V_5 \; V_6^0 \; V_{17}^0 \; V^0 + V_9^1 \; V_{19}^2 + V_{20}^0$

Fig 1 Matrix of bivariate distribution of scores V_b^a=bth variable a=1 2 3 nth *intervals of a new score into which the* original values of the bth variable can be *partitioned* O—absence of new score

for absence of involvement in a particular anatomic segment, and nines for no information Numbers one through eight were used for the subdivisions of the anatomic segments involved

The choice of the degree *of discreteness in defining anatomic sites or segments* and in subdividing them, depends on many factors It is usually governed by conventionally defined anatomic parts of organs or regions It also depends on how well the clinicians have been able to distinguish involvement between the variously defined sites by physical examination and through routinely performed laboratory *procedures Divisions that are too fine lead to unreliable* and over lapping measurements and to undesirable excessive dilution of the material Divisions that are too rough produce loss of important information

The choice of properties and categories for analysis is an intellectual exercise requiring the highest order of clinical judgment, as essential in this method *for constructing staging systems as in any other* It can only be made by physicians who are exceptionally well grounded in oncology and particularly familiar with the cancer entity under consideration However the decisions made are provisional and in this method are subjected to quality control evaluation Furthermore, the method establishes the *hierarchy of the properties selected in a way independent* of clinical judgment

Computation Analysis of data on cards were undertaken on the 360—40 IBM computer Although manual procedures of data sorting, manipulation and

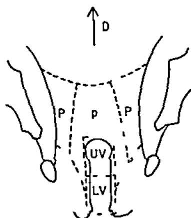


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Table 1

**Mathamedical anatomical classification of recurrent endometrial carcinoma — For symbolic concepts refer to* FEINSTEIN (1967)

Diagram	Definition	Symbolism		Survival
		STAGE I		
	Upper Vagina	$UV\ \overline{LV}\ p\ \bar{P}\ \bar{D}$		45/55 (82)
		STAGE II A	SUBTOTAL	16/30 (53)
	Pelvis (not fixed)	$\overline{UV}\ \overline{LV}\ p\ \bar{P}\ \bar{D}$		2/3 (66)
	Lower Vagina	$\overline{UV}\ LV\ p\ \bar{P}\ \bar{D}$		6/11 (55)
	Upper Vagina and Pelvis (not fixed)	$UV\ \overline{LV}\ p\ \bar{P}\ \bar{D}$		8/16 (50)
		STAGE II B	SUBTOTAL	7/30 (23)
	Upper Vagina and Pelvis (fixed)	$UV\ \overline{LV}\ (p\ P\ \ p\ P)\ \bar{D}$		4/9 (45)
	Lower Vagina and Pelvis (not fixed)	$\overline{UV}\ LV\ p\ \bar{P}\ \bar{D}$		2/6 (33)
	Pelvis (fixed)	$\overline{UV}\ \overline{LV}\ (p\ P + p\ P)\ \bar{D}$		1/9 (11)
	Upper and Lower Vagina	$UV\ LV\ p\ \bar{P}\ \bar{D}$		0/4 (0)
	Upper and Lower Vagina and Pelvis (not fixed)	$UV\ LV\ p\ P\ D$		0/4 (0)
		STAGE III	SUBTOTAL	5/34 (5)
Various	D t t Group	$(UV\ LV\ \ UV\ \overline{LV})\ p\ \bar{P}\ D\ \ (UV\ \overline{LV}\ \ \overline{UV}\ LV + UV\ LV)$ $\cdot(p\ \bar{P}\ \ p\ P\ \ p\ P)\ D + \overline{UV}\ \overline{LV}\ (p\ \bar{P}\ \ p\ P\ \ p\ P)\ D\ \ \overline{UV}\ \overline{LV}\ p\ \bar{P}\ D$		
			TOTAL	73/149 (49)

* Not all types of anatomical configurations are shown
** For abbreviations refer to Fig 2 Symbol + indicates either or or both symbol both and symbol $\overline{UV}$ *negation i e upper vagina not involved*
*** Crude 32 month survival rate

The selection procedure operable in columns, rows, or both, can be applied to all cases to be included in the table However, in addition to manual selection, this can be done automatically on all combinations of a specified number of variables For example, in a set of four binary (yes or no) variables, tabulations of 16 types of combination of cases are produced

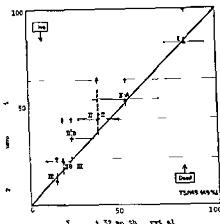


Fig 3 Assessment of constructed staging system in recurrent endometrial carcinoma

One or more variable values can be transformed prior to the selection and tabulation operations In the analysis of these cancer data the most frequently used transformation procedures were rank logarithms dates to time additions subtractions multiplications and divisions of one or more variables One or more of these procedures can be applied to the same variables in succession and in any sensible order

Many subroutine procedures for statistical calculations may be initiated by the main program The ones most frequently employed were (1) the survival table construction (2) N × M and 2 by 2 chi square tests (3) Fisher s exact test (4) Wilcoxon rank sum test (5) unpaired t test (6) normal correlation and linear regression (7) rank correlation (8) analysis of variance (9) the Gehan test for comparison of response curves with censored data and (10), on appropriate occasions the Mann Whitney test

A large number of search types of combinatorial analyses is made possible by this computer program The amount of tabulated and statistically analyzed data results may become extensive almost excessive although tables that contain none or less than a minimum specified number of counts can be excluded In this particular study the computation procedure yielded approximately fifty pounds of tabulated data in order to adequately search for the proper definitions of each level of stage

Results

A schematic diagram of the subdivisions of anatomic sites which were found to have individual prognostic distinctions is presented in Fig 2 The finer ones initially defined in the methods section yielded too many kinds of subgroups when

they were used singly or in combinations In addition, many of them were not represented by the case material, many subsets being found empty These latter were eliminated and those remaining were grouped together according to similarities in survival rates after detection of recurrences The distant group contains many subgroups of cases with various patterns of spread all of which were indistinct However, once the presence of cancer was established outside the pelvis, the prognosis was predictably poor

A summary of representative patterns of recurrences is given in Table 1 The arrangement is in decreasing order of crude 32 month survival rates This rate has been substituted for the one of 5 year survivals, for reasons that will be taken up in the discussion of Fig 3

The stage I level includes only cases of pure upper vaginal recurrences with a 32 month survival rate of 82 % Stage II includes cases with lower vaginal or pelvic recurrences The survival rate is 39 % This stage has been divided into two levels at a point where the subgroup with the highest rate contained cases with pelvic fixation Because of the limited number of cases this point of demarcation was chosen more from a morphologic than a statistical context so that this choice had to be somewhat arbitrary It is seen that stage II A cases are those with central pelvic recurrences with or without upper vaginal lesions having a 32 month survival rate of 53 % Stage II B cases are those exhibiting pelvic fixation or manifesting lower vaginal recurrences in combination with lesions at two or more other pelvic sites the survival rate in this stage is 23 % It is of interest to note that the cases in stage II B categories with lower vaginal involvement have low survival rates

The stage III level includes all cases with spread of cancer outside the pelvic region The survival rate in this group is 15 %

A method of assessing the effectiveness of the staging procedure is illustrated in Fig 3 The survival rates of cases with the various stage levels were calculated at a time after the initial reference point (i e the time of clinical detection of recurrence in this study) when half of the total initial population had died

Referring to the plotting of percentage cumulative stage incidence as the ordinate, and percentage survival determined at this time as the abscissa, the total sample population may be conceived as being distributed uniformly in the square area formed by the coordinates and separated by a diagonal between them into 'alive and 'dead patients The alive patients would be distributed above the diagonal and the dead patients below Due to the limits of sample size, a 49 % survival rate was the best possible calculable half population survival rate in this example

The crux of the problem of staging then is the proper selection of definitions for each stage level so that cases so grouped have survival rates at each of the

Table 2

Results of radiotherapy in stage I recurrent endometrial carcinoma

Treatment	32 month survival
Radium	20/24 (83)
Roentgen	5/8 (63)
Roentgen and radium	13/15 (87)

Table 3

Results of radiotherapy in stage II recurrent endometrial carcinoma

Anatomic subdivisions	32 month survivals		
	Radium	Roentgen	Roentgen and radium
Lower part of vagina	2/4 (50)	2/4 (50)	2/3 (67)
Lesions with pelvic spread (not fixed)		6/16 (37)	6/13 (46)
Lesions with pelvic spread (fixed)		1/9 (11)	4/9 (45)
Total	2/4 (50)	9/29 (31)	12/25 (48)

levels parallel to and close to the diagonal The closeness then to which the stage levels approximate the diagonal is a rough measure of the efficiency of the staging system evolved Furthermore each staging level should be distinctly separated from the others and encompass a good part of the diagonal It should be noted that if survival rates for each of the stage levels are computed at a time point when the total sample population rate is greater than 50 % the plotted values will be shifted to the right of the diagonal The diagonal will be curvilinear with a central sag toward the right hand corner dividing the area into alive and dead proportions and vice versa Hence the use of total rates differing greatly from 50 % complicates this procedure and necessitates a formal expression of the cumulative incidence of stage levels and percentage survival values

The cross or double cross plots indicate the cumulative incidence of stage against survival rate whereas the triangle or diamond plots give the midpoint stage interval of cumulative incidence against rate The horizontal lines are the estimates of the 95 % confidence intervals The vertical dotted lines divide the subsample population for each of the stage levels into alive and dead subjects

All stages except stages II B and III are prognostically distinct ($p < 0.02$) Stages I II A and III lie close to the diagonal but stage II B is somewhat

Table 4

Comparison of three staging systems in carcinoma of the corpus uteri

Anatomic sites	International (1963)	Gusberg (1966)	Hirabayashi (1968)
Corpus	I	—	—
Normal	—	I (< 9 cm)	
Enlarged	—	II (9—10 cm)	I (< 8.5 cm)
Markedly enlarged	—	III (> 10 cm)	II (> 8.5 cm)
Cervix	II		III
Parametria, Pelvic wall	III	IV	IV
Distant	IV		

deviant. The best prognostic divisions are stages I, II A, and II B plus III, but for treatment purposes it would be better to combine the cases with pelvic extensions (stages II A plus II B) or to keep stage II B as a separate category, as in Table 1. The latter system is also prognostically distinct when stages II A and II B are combined. However, it is not as good as the former, as demonstrated by the tendency of the survival rate of this combined group to overlap that of the stage III group.

Since survival (crude, tumor free or other types) is the known measure of the success of treatment procedures, it is necessary to equalize powerful prognostic factors, such as stage, among the treatment groups before the comparison. If this equalization is omitted, a heterogeneity of stage levels will almost inevitably lie between or among the treatment groups, which if not taken into account, must lead to a misinterpretation of the relative efficacy of the various treatment methods that are being compared. That such misinterpretation is all too common is readily evidenced by inspection of the current medical literature.

Tables 2 and 3 are presented to demonstrate the applicability of this or a similarly constructed staging system for the comparison of treatment methods. If it is accepted that duration and possible morbidity of roentgen therapy are undesirable compared to vaginal radium treatment, then the latter alone would appear to be the best modality for stage I lesions, whereas the need for external therapy is indicated when this neoplasm has extended into the pelvic regions. The controversy relative to the radiologic and surgical management in recurrent corpus carcinomas has been discussed elsewhere (BADIB et coll. 1969).

Since other staging systems for recurrent carcinoma of the corpus uteri were not encountered in the literature, those previously established for primary cancers of this organ were utilized for the purpose of demonstration. The three

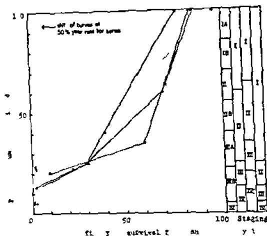


Fig 4 Assessment of staging systems in primary carcinoma of the corpus uteri Roswell Park Memorial Institute Series 1945—1964 345/563 (61 %)

systems chosen for evalution are presented in Table 4 Data from a recent publication comparing three systems of staging applied to a single series of cases (HIRABAYASHI et coll 1968) have been employed

A total of 136 out of 766 cases were excluded in the cited study prior to the calculation of survival rates because they had succumbed from other causes Unfortunately this removal of an unknown proportion of cases five or more years after the diagnosis tends to produce a bias toward lowering the survival rates more than when these rates are calculated on a time at risk basis and are then corrected for non-cancer deaths It also might have been somewhat more desirable to use the percentage survival values at a time when half of the total population at risk had expired i e some time after five years rather than those at five years The first bias tends to shift the plotted values to the left while the second causes a shift to the right For these reasons the survival rates required for this method of assessment cannot be ascertained from these data However this uncertainty naturally does not interfere with the use of these data for a demonstration of the method described in this report As mentioned above when a staging system approaches an ideal system the plotted values of each level should be parallel to the diagonal (Fig 3) or to some curvilinear off diagonal dividing line distinctly separate from each other

The international system (See *Annual* Report on the Results of Treatment of Carcinoma of the Uterus 1958) approaches proximity to the diagonal band at stage levels II III and IV but the latter two are prognostically inseparable (Fig 4) All three measure only the lower third of the diagonal Its stage I group includes disproportionately large portion of the patient population with

most of the survival information lost within it. It is the most deviant of the three GUSBERG's (1966) and HIRABAYASHI's (1968) systems tend to follow the downward curvilinear diagonal line dividing the total sample into 60 % alive and 40 % dead patients. However, in the former system the gap between stages *III* and *IV* is relatively large, whereas in the latter the gap between II and III is large. It would *appear therefore that an opportunity exists to make further* worthwhile improvements in the existing staging systems for primary corpus carcinoma. For instance, by including in a staging system the variables, uterine cavity depth and histologic grade, along with the currently used anatomic variables, the staging system thus evolved might more closely approach an ideal one, as assessed according to the scheme now proposed.

Another consideration involves the use of prognostic variables with continuous scores, e.g. cm diameter of lesion, cm depth of uterine cavity, years of age. These should ideally be demarcated into intervals by determining points at which the survival rates change, before incorporating them with the various anatomic components. For example, in primary cervix cancer without extension, tumor size in terms of small and large diameter should be demarcated by a point separating them into centimeter intervals predicting the greatest difference in rates possible.

Discussion

The primary concept of measurement in the clinical staging of cancer is the prediction of outcome of the disease. To develop this concept, knowledge of the manner of growth and of direction of spread of the cancer entity must be available. Correlative clinicopathologic studies may be used to establish or indicate *that the important clinical variables in defining staging* systems for each histologic type of cancer are (1) the anatomic sites of involvement, (2) tumor size, (3) depth of invasion and (4) histologic grade. Other known variables seem less important but may be added to modify the system, e.g. age, symptoms, duration, treatment, concurrent disease as appropriate.

Although the prognostic significance is known individually for many of the clinical as well as of other types of variables, little is known about the degrees of their correlation or interdependencies. In a highly structured multivariate system, as *in human beings, there are variables exhibiting* a wide range of correlations, from complete independence to complete dependence. Well correlated variables contribute only redundant information on prognosis so that they should be combined into a few clusters.

Partially correlated variables manifest their influence in varying degrees through others, so that those with the strongest effects on prognosis should be

removed from those with less in decreasing order of effects until those with little or none are encountered The latter should be eliminated In this way it is possible greatly to reduce the numbers of variables affecting prognosis permitting an easier handling of those remaining and minimizing the danger of mixing those with different levels of prognostic distinctions

There is danger in simplifying staging systems on the basis only of practicability or perhaps merely to incorporate large numbers of cases in each stage level selected Such lumping together of unknown proportions and unidentified content of artificially defined staging variables may yield precise but useless generalities which may well be inaccurate and valueless when applied to individual patients The stipulation made in connection with changes in the staging systems for various types of cancer in the past suggest that some such considerations may have induced these changes

Even in the time honoured highly successful international classification of the stages of carcinoma of the uterine cervix certain discrepancies are evident For example involvement of the bladder or rectum or both without reference to other sites cannot possibly correlate with other variables cited for inclusion of cases in the stage IV category Cases of local cervical lesions with bladder or rectal involvement at the time of diagnosis are likely to do much better than those with bilateral pelvic wall fixation and such involvement Treatment wise these cases should therefore also be placed in different categories The former may more likely be amenable to pelvic exenteration procedures with relatively good results whereas the latter may not

The separation and combination of objects for classification is sometimes referred to as lumping and splitting The initial splitting is mandatory in clinical analyses without these initial divisions the clinician lumps a melange of unknown entities Having split he can always lump later but he then has the major scientific advantage of knowing what he lumps and why (FEINSTEIN 1967) i e after he has knowledge of the distinctiveness of the objects

Concepts of definitions for the measurement of prognostic variables vary for different types of cancer originating in different organ systems Regional lymph nodal involvement cannot be assessed with the same degree of clinical validity in gynecologic cancers as in those of the head and neck This is evident in the TNM system initially developed for the latter but recently proposed for cancers of the uterine cervix

In contrast to head and neck cancer nearly all of the prognostic information in carcinoma of the cervix uteri is contained in the T categories and little or none in the N categories while M1 is entirely contained in stage IV B Furthermore the Nx category has not been ordinarily sought for or discovered in cancer of the cervix in most clinical situations It will be interesting to observe how well

most of the survival information lost within it It is the most deviant of the three *Gusberg's (1966)* and *Hirabayashi's (1968)* systems tend to follow the downward curvilinear diagonal line dividing the total sample into 60 % alive and 40 % dead patients However, in the former system the gap between stages III and IV is relatively large, whereas in the latter the gap between II and III is large It would appear therefore that an opportunity exists to make further worthwhile improvements in the existing staging systems for primary corpus carcinoma For instance, by including in a staging system the variables, uterine cavity depth and histologic grade, along with the currently used anatomic variables, *the staging system thus evolved might more closely approach an ideal* one, as assessed according to the scheme now proposed

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Discussion

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this system of staging works out in the actual clinical setting where the credibility of the TNM system must be eventually determined

The method described is believed to provide a logical medical taxonomic type (SNEATH 1964) approach to cancer staging It is further believed that if utilized, it could reduce the production of numerous incompatible staging schemes derived by trial and error, the latter have probably been developed due to the classification with prior systems that have proved of little utility in the clinical setting *The method proposed may well* permit experienced clinicians to design creditable staging systems allowing for simplicity and practicability by the logical lumping of subsets of cases with similar prognostic as well as clinical distinctions

Acknowledgement

The authors take this opportunity of thanking Bernard Hanes for his help in suggesting the mechanics that might be applicable This study was supported *in part* by the USC Medical School General Research Fund and by Social Security Research Contract 467

SUMMARY

An objective nonempirical approach to the construction of clinical cancer staging systems is described The method has been utilized as a pilot project to develop a staging system for recurrent endometrial carcinoma following total hysterectomy in a series of 149 case The system so devised has been evaluated and found to be accurate in terms of prognosis and accordingly an effective means of comparing treatment methods

ZUSAMMENFASSUNG

Ein objektives nicht empirisches System der Einteilung von Krebs in verschiedenen Stadien wird beschrieben Versuchsweise wurde dieses System zur Einteilung von 149 Fallen von recidivierendem Karzinom des Endometriums nach vorhergehender Resektion des Uterus angewandt Die neugeschaffene Stadieneinteilung wurde kritisch uberpruft und zeigte sich prognostisch als zuverlasslich und nutzlich im Vergleich verschiedener Behandlungsmethoden

RÉSUMÉ

Les auteurs decrivent une methode objective et non empirique pour etablir un systeme clinique de classement de cancer par stades Cette méthode a été utilisée comme projet pilote pour mettre au point un systeme de classement des recidives de carcinomes de l'endometre apres hystérectomie totale sur 149 cas Ce systeme a été mis à l'epreuve et considere comme precis en ce qui concerne le pronostic et par consequent considere comme un moyen efficace de comparaison des methodes de traitement

Table 1

Experimental conditions and ^{90}Sr doses employed

Dose of ^{90}Sr in µCi g body weight	Total number of mice investigated	Number of mice killed in groups of five at given intervals	Last day for sacrifice	Number of mice that died naturally
Sacrificed mice				
1 6	120	65	300	50
0 8	121	75	360	46
0 4	122	95	480	27
0 2	122**	103***	540	17
Control	95	94****	570	1
Mice used for cell distribution analysis				
0 8	20	20	60	0
0 4	60	50	300	0
0 2	20	20	60	0
Control	50	50	300	0

Of these animals five and two were lost during the experiment only three*** and four**** animals respectively were sacrificed in the last test group

The extent to which different doses of ^{90}Sr affect the functional relations between the thymus spleen and bone marrow will also be considered

Material and Methods Four groups of CBA male mice 75 days old were treated intraperitoneally with different doses of $^{90}Sr(NO_3)$ A group of 95 untreated animals were used as controls Five mice from each group were selected at random and sacrificed at intervals of 7 14, 21 and 30 days after injection of ^{90}Sr and then at monthly intervals until all the surviving mice in each series had been used up

The quantitative relation between cells in the bone marrow, spleen and thymus was studied by distribution analysis One hundred mice were separated into three groups and then injected with 0 2 0 4 and 0 8 µCi/g body weight respectively Fifty mice were used as untreated controls Five mice from each of these groups were killed after 7 14 and 21 days and after 2 months In addition five mice from the control groups and from the 0 4 µCi group were investigated at 3 4 5 7 9 and 10 months Experimental conditions and the doses of ^{90}Sr employed are recorded in Table 1

FROM THE DIVISION OF RADIOBIOLOGY (DIRECTOR DOCENT A NILSSON), THE RESEARCH INSTITUTE OF NATIONAL DEFENCE, SUNDBYBERG, SWEDEN

PATHOLOGIC EFFECTS OF DIFFERENT DOSES OF RADIOSTRONTIUM IN MICE

Changes in the haematopoietic system

by

AGNAR NILSSON

The early and late effects of ^{90}Sr on the haematopoietic system have often been investigated (2, 3, 7, 8, 9, 10 12, 15, 19, 21) but they are in many respects poorly understood since the time dependent distribution in the tissues and the biologic behaviour of ^{90}Sr are very complex Most of the tissues are initially subjected to short irradiation, before the radiostrontium is almost completely confined to the skeleton The marrow will be particularly exposed but the irradiation dose will differ considerably depending on size and shape of the bone and the rate of redistribution of the ^{90}Sr in the different parts of the skeleton The extraskeletal parts of the haematopoietic system may on the other hand to some extent be directly, but more and more indirectly, influenced by the successively decreasing regeneration capability of the bone marrow after protracted irradiation

Some of these aspects have earlier been investigated and reported on (16, 17) The present paper deals with the quantitative and qualitative as well as the time dependent effects of various doses of ^{90}Sr on the haematopoietic system

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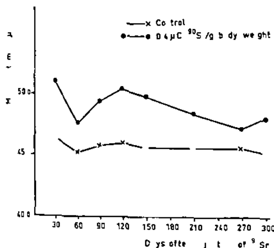


Fig 2 Mean volume of erythrocytes in peripheral blood in relation to time after injection of ^{90}Sr Persistent macrocytosis

as possible the ^{90}Sr induced quantitative variations in the different cell populations Parts of the thymus and spleen were suspended in homogenization tubes containing a relatively particle free balanced salt solution After cutting both ends of the femur the marrow was forced out by compressed air and handled in the same way as the thymus and spleen All the organ suspensions were adjusted to contain between 10^4 and 3×10^4 cells per milliliter The peripheral blood was also investigated in suspensions of 1 2 000

The cell volume distribution was studied with an electronic counter (Coulter Counter Model B) equipped with an automatic particle size distribution analyser (Coulter Plotter Model J) The principle and operation of this instrument have been described by BRECHER et coll (1962) The instrument was calibrated with rat erythrocytes and essentially monosized Ragweed Pollen for the absolute cell volume determination

The cells investigated were divided into small cells of size 40 to 100 μ^3 moderate sized cells 100 to 200 μ^3 and large cells 200 to 500 μ^3 The limits for the three groups of cells were approximate but chosen as far as possible to represent the main bulk of morphologically defined cell entities, they seem to agree fairly well with the results obtained by HAOT et coll (1967) The reason for setting the upper limit for the small cells at 100 μ^3 was that the main part of these cells consists of erythrocytes with a mean volume of between 40 and 50 μ^3 (range 20 to 140 μ^3) The influence of leukocytes on the volume distribution of the peripheral blood could be designated as insignificant The moderate sized cells were chosen within limits mainly representing the lymphoid cell elements Their mean volume was between 120 and 140 with a range of 100

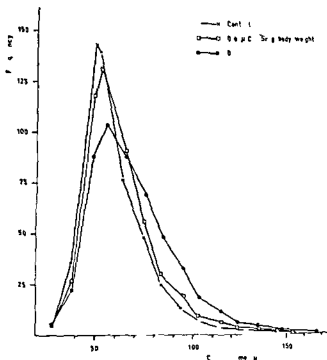


Fig 1 Cell volume diagram (Coulter counter) of peripheral blood with macrocytosis 21 days after injection of ^{90}Sr

The mice before sacrifice were anaesthetized and blood samples were obtained with a Pasteur pipette from the medial venous plexus of the eye Total counts of leukocytes and thrombocytes were made The haemoglobin values were expressed in gram per 100 ml blood Blood smears for the differential counts were dried in air, stained with May Grunewald Giemsa, and 200 cells were counted in each smear

For the histologic investigation, the femora, tibiae and humeri as well as the pelvic bones, the spine, thymus, spleen, mesenteric lymph nodes, liver, kidneys and the brain were fixed in Stieve's fluid These tissues were prepared according to conventional histologic methods and stained by the van Gieson method, haematoxylin and eosin, Lillie's azure eosinate and PAS according to Hotchkiss

The bone marrow in the distal, proximal and diaphysial parts of each pair of the long bones and in the cervical, thoracic, lumbar and sacral spines, as well as in the regions of the tuber sacrale, tuber ischii, ilia, acetabula and basis cranii were analysed with respect to their degree of cellularity The cellularity of the marrow was classified according to an arbitrary scale from 0 (aplastic marrow) to 5 (normal marrow) The cellularity for a group of five mice was expressed as the mean obtained from 155 measurements

The analysis of the cell volume distribution was made in combination with the investigation of the histologic features in order to demonstrate as objectively

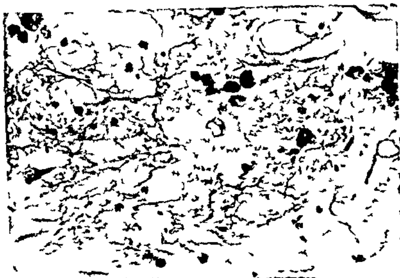


Fig 3 Haemosiderin loaded macrophages in aplastic and necrotic bone marrow of femur 300 days after injection of 1 6 μCi ^{90}Sr/g body weight van Gieson × 500

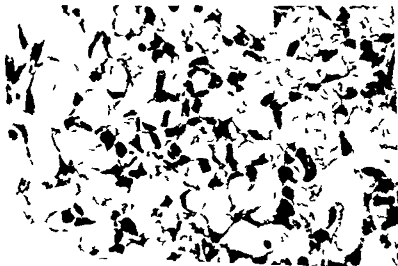


Fig 4 Proliferation of reticular cells in aplastic bone marrow of diaphysis of femur 300 days after injection of 0 8 μCi ^{90}Sr/g body weight

to 200 μ^3 The 'large cells' were mostly various types of blasts (usually over 200 μ^3)

Results

Peripheral blood A significant and persistent leukopenia developed in all the dose groups at 7 days Expressed as the mean for the whole experimental period the following dose related levels of leukocytes were observed

1 687 7 ± 153 2 (range 1 072—2 970) in the 1 6 μCi group
3 063 7 ± 181 4 (range 2 112—4 548) in the 0 8 μCi group
4 223 5 ± 213 3 (range 2 980—5 972) in the 0 4 μCi group
4 837 9 ± 238 2 (range 2 820—6 948) in the 0 2 μCi group
8 383 3 ± 247 8 (range 6 836—10 378) in the control group

A shift in the differential counts also occurred The mean percentage distribution of granulocytes in the ^{90}Sr treated groups was over 30 % (range 25 6—38 4 in the 1 6 μCi group, 29 0—62 8 in the 0 8 μCi group, 23 6—43 8 in the 0 4 μCi group and 25 6—36 0 in the 0 2 μCi group) The control value was 19 9 % (range 15 6—29 2) A corresponding and persistent lymphopenia was observed

The haemoglobin values in all the dose groups reached a minimum within 14 days A slight recovery then occurred but normal values were never regained in anyone of the groups The mean values (g/100 ml blood) for the whole experimental period were as follows

11 2 ± 1 95 (range 9 9—12 5) in the 1 6 μCi group
11 7 ± 1 52 (range 10 4—12 4) in the 0 8 μCi group
12 1 ± 1 70 (range 10 7—13 4) in the 0 4 μCi group
12 5 ± 1 02 (range 11 4—13 2) in the 0 2 μCi group
13 3 ± 2 14 (range 11 6—15 6) in the control group

Marked macrocytosis (Fig 1) accompanied by an increase of polychromatic erythrocytes were present in the peripheral blood in all the dose groups investigated (0 2, 0 4, 0 8 μCi ^{90}Sr/g body weight) as early as at 7 days The macrocytosis persisted when investigated for a period of up to 300 days after the injection of 0 4 μCi ^{90}Sr/g body weight (Fig 2)

The thrombocyte counts reached their minimum values at 14 days in all the dose groups but thereafter a rapid increase to normal or even higher values occurred The following mean values were obtained

532 000 ± 51 900 (range 247 800—771 000) in the 1 6 μCi group
536 000 ± 50 500 (range 225 600—854 800) in the 0 8 μCi group
549 500 ± 39 400 (range 196 000—984 000) in the 0 4 μCi group
596 100 ± 40 800 (range 259 600—885 000) in the 0 2 μCi group
534 000 ± 19 000 (range 384 400—720 750) in the control group



Fig 3 Haemosiderin loaded macrophages in aplastic and necrotic bone marrow of femur 300 days after injection of 1 6 μCi ^{90}Sr/g body weight van Gieson × 500

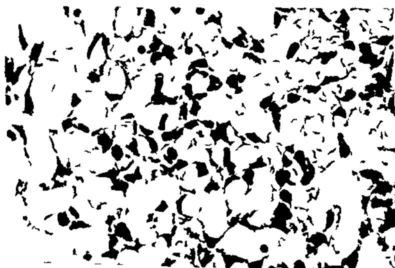


Fig 4 Proliferation of reticular cells in aplastic bone marrow of diaphysis of femur 300 days after injection of 0 8 μCi ^{90}Sr/g body weight

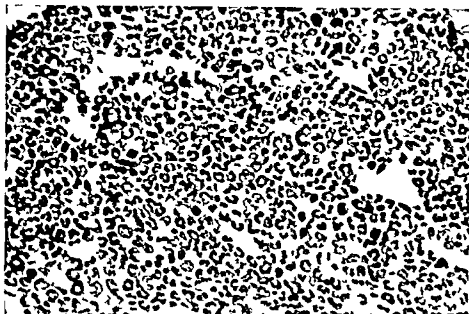


Fig. 5. Bone marrow of femur. Granulocyte proliferation 150 days after injection of 0.2 µCi ^{90}Sr/g body weight. van Gieson × 500.

Bone marrow. The histologic changes differed quantitatively but were principally of the same type in all the dose groups. Dilatation and hyperaemia of the medullary sinusoids accentuated with increasing dose. Increased amounts of fatty tissue appeared, more abundant and earlier in the two higher dose groups than in the others. Appreciable amounts of fat appeared in the 1.6 µCi group as early as at 7 days.

Destruction of the sinusoids with formation of localized blood lakes was more extensive in the lower than in the higher dose groups. This was the case particularly at 270 days in the 0.4 µCi group. Thromboses in different stages of organisation were also a common feature long after the injection of ^{90}Sr, particularly in the 0.2 µCi group at 390 days but also in the 0.4 µCi group. They appeared most frequently in the diaphysial part of the marrow of the long bones.

Marrow necrosis was present, above all in the femur, in all the dose groups, but earlier (Fig. 3) in the 1.6 µCi group (210 days) and the 0.8 µCi group (360 days) than in the others. In many aplastic or markedly hypoplastic marrows, only reticular cells remained, or were increased, or were in a state of proliferation (Fig. 4). This occurred particularly in mice given 0.4 µCi/g body weight. These cells have in earlier work (Sundelin & Nilsson 1968) been shown to start the development of predominantly fibroblastic osteosarcomas

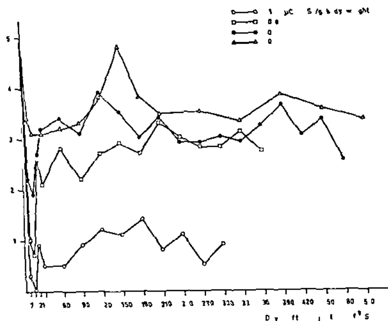


Fig 6 Bone marrow cellularity in relat on to dose and time after injection of ^{90}Sr according to an arbitrary scale 0 equals aplasia 1—4 varying degrees of hypoplasia and 5 normal marrow cellularity each point on the diagram represents the mean marro cellularity from 155 estimations

Five mice were investigated in each of which the marrow cellularity was estimated in the distal diaphyseal and proximal parts of the femora tibiae and humeri respectively in each of the two pelvic bones at the tuber ischii tuber sacra e the ilium and the acetabular region in the spine in one marrow cavity in each of the cervical thoracic lumbar and sacral vertebrae and in the head the base of the cranium

The 1 6 μCi group was the only one in which early fibrosis (before 30 days) was detected This fibrotic tissue in one instance contained large cells with cytoplasmatic non metachromatic diastase resistent PAS + granules Almost total destruction of all types of marrow cells occurred in the 1 6 μCi group during the initial phase Small islands of erythroblastic elements containing immature polychromatic erythrocytes could be detected at about 14 days Prominent anisochromia macrocytosis and poikilocytosis were characteristic of these cells many of which were migrating through the capillary walls into the circulation A *shift* in the relative proportions between the granulocytic and erythrocytic eries was detected 21 days after ^{90}Sr administration and from that time onwards a predominance of the granulocytic series took place

In the other groups the erythrocytic series showed a tendency to predominate

Table 2

Distribution of mice (per cent) in relation to ⁹⁰Sr dose and bone marrow cellularity

Dose group μCi/g	Bone marrow cellularity					
	Aplastic 0 0—0 5	Markedly hypoplastic 0 6—2 5	Moderately hypoplastic 2 6—3 5	Slightly hypoplastic 3 6—4 5	Normal 4 6—5 0	Hyperplastic >5
1 6 n 65	32 3	67 7	0 0	0 0	0 0	0 0
0 8 n 75	0 0	45 4	52 0	0 0	2 7	0 0
0 4 n 95	0 0	19 0	68 4	8 4	3 2	1 1
0 2 n 103	0 0	0 0	60 0	32 9	5 7	1 4

in regeneration during a short period (before 30 days) The granulocytic series started to dominate at 90 days in the 0 8 μCi group and after about 120 to 150 days in the others This granulocytosis was temporary in the 0 2 μCi group and ended after about 360 days

Marked hyperplasia, particularly in the bone marrow but also in the spleen, of hardly any other cells than those representing the granulocytic series, occurred in several instances, especially in the lower dose groups (Fig 5)

Megakaryocytes were more abundant than normally in the 0 4 and 0 2 μCi groups, and this particularly between 330 and 390 days In the 1 6 μCi group, megakaryocytes re appeared after the initial period but never attained their normal number

An abundance of mastocytes were seen in some instances in the 0 8 and 0 4 μCi series in the bone marrows and in most lymphoid tissues, including the thymus

Quantitative analysis The effect of the various doses of ^{90}Sr on the bone marrow cellularity among the sacrificed mice is recorded in Fig 6 The minimum values were observed in all series at 14 days, the cellular depletion increasing with increasing dose The marrow depletion within each group was initially of about the same magnitude in the various marrow cavities investigated but the start and degree of marrow regeneration differed greatly in the various parts

The percentage distribution of sacrificed mice in relation to dose and their mean bone marrow cellularity is shown in Table 2 Six out of fifty mice in the

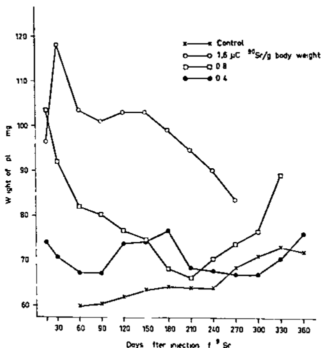


Fig 7 Weight of spleen in relation to dose and time after injection of ^{90}Sr

1 6 μCi group died naturally of acute and four of chronic aplastic anaemia Chronic aplastic anaemia occurred in the 0 8 μCi group in one out of forty six and in the 0 2 μCi group in one out of seventeen mice

The Coulter analysis revealed a prominent and persistent increase of small cells mostly representing the engorgement of the sinusoidal space by erythrocytic elements. The degree of hyperaemia seemed to be initially related to dose, i e the degree of hyperaemia increased with increasing dose Moderate sized cells representing lymphoid elements were considerably reduced and never regained normal values nor did the large cells representing blasts

Marrow regeneration Topographically the start and intensity of regeneration varied and proceeded according to the bone in which the marrow was located The marrow of the femurs in the 1 6 μCi group presented little evidence of regeneration apart from scattered small foci of dividing cells and was practically aplastic until the death of the animals. On the other hand considerable regeneration took place focally in the marrows of the tuber sacralae tuber

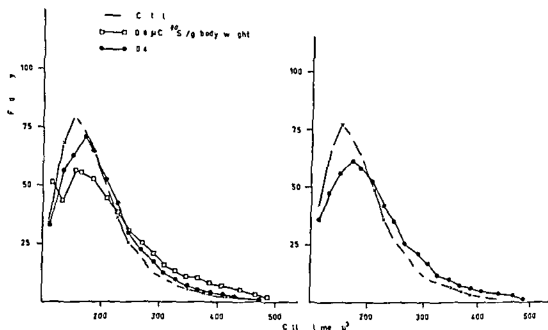


Fig 8 Cell volume diagram (Coulter counter) for spleen 21 days (left) and ten months (right) after injection of ^{90}Sr Dose related and persistent lymphocyte depletion

ischii, the thoracic vertebrae as well as in the sternal marrow, and reached its peak at about 180 days after the injection of the ^{90}Sr Cells of the granulocytic series predominated in these islands of regeneration

Also in the other groups the marrow of the femur was more damaged than other parts The most active marrows were present at the same sites as in the 1 6 μCi group but the regenerative capacity was greater and related to dose, reaching its peak in the 0 8 μCi group at 210 days, as compared to between 120 and 150 days in the other groups The decreased marrow activity in the femurs was however compensated for predominantly in the thoracic vertebrae and sternal marrow

Spleen Changes in the weight of the spleen are illustrated in Fig 7 In spite of a heavy loss of lymphatic tissue in the higher dose groups and a moderate loss in the two lower dose groups (Fig 8), a most significant increase in weight persisted over a long period, this was caused by hyperaemia and a heavy erythro-, megakaryocyto- and granulocytopoiesis (Fig 9) The degree of lymphoid depletion depended upon the dose

Histologically the findings largely agreed with those described in an earlier paper (16) It should however be noted that in the 1 6 μCi group the increased extramedullary blood formation, which was initially dominated by the erythroid

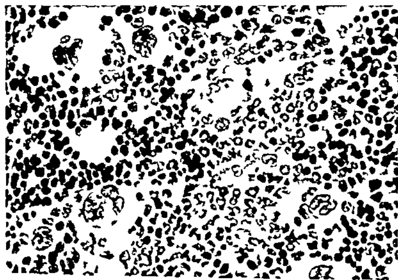


Fig 9 Appearance of cells of spleen two months after injection of 1 6 μCi ⁹⁰Sr/g body weight Extra medullary haematopoiesis of all types of cells H—E × 500

series was successively and after about 200 days completely replaced by the granulocytic cell series.

Extramedullary haematopoiesis in the 0 8 μCi group was marked but in contrast to the 1 6 μCi group erythroid elements were usually more abundant in the process of regeneration than the granulocytic series of cells

Megakaryocytopoiesis was much more prominent in the two lower dose groups than in the two others

Thymus Changes in the weight of the thymus in the control groups and in the different ⁹⁰Sr series are illustrated in Fig 10 The loss of weight and enhanced rate of involution are dose dependent and as shown by the cell volume distribution analysis predominantly caused by a depletion of lymphoid cells (Fig 11) This lymphoid depletion was not detectable histologically

Discussion

A general depletion of all haematopoietic tissues occurred in all the dose groups but only in the 1 6 μCi group was a high frequency of aplasia recorded among the sacrificed animals (see Table 2) In this group the mice also died naturally from acute aplastic anaemia

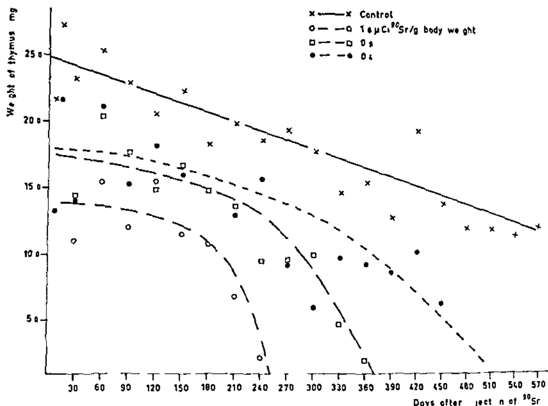


Fig 10 Weight of thymus in relation to dose and time after injection of ^{90}Sr

The weight of the thymus diminished increasingly with enhanced dose and the weight of the spleen increased progressively It is obvious from the cell volume distribution diagram that the diminished weight of the thymus is caused by a constant depletion of lymphocytes, predominantly in the cortical zone Persistent lymphoid depletion occurred also in the spleen but the increased weight was brought about by hyperaemia and an extramedullary diffuse haematopoiesis, the intensity of which mainly mirrored the degree of damage to the bone marrow, in which practically all cell types were depressed, and the degree of this depletion was largely related to dose

The destruction of the sinusoids in the bone marrow may have been directly related to the effect of irradiation since it was most widespread at the highest dose level Vascular disorders may have been indirectly involved, as was indicated by the more frequent occurrence of blood lakes and necrosis in conjunction with thrombosis in the lower dose groups It should be noted in this connection that thrombocytosis predominated, particularly in the lower dose groups

In the regeneration of bone marrow a general pattern could be distinguished

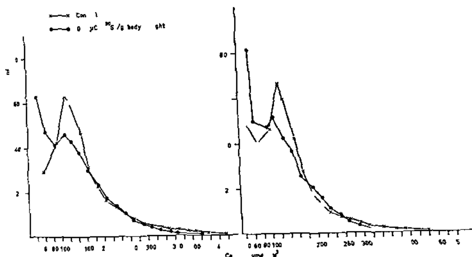


Fig 11 Cell volume diagram (Coulter counter) for thymus one month (left) and ten months (right) after injection of ^{90}Sr Persistent lymphocyte depletion

irrespective of the dose Topographically this regeneration was mainly determined by the redistribution and amount of ^{90}Sr released and by the size and shape of the bone The most active locations were the thoracic vertebrae, the sternum and certain sites in the pelvic bones The evidence of regeneration was in significant in the 1 6 μCi group with the exception of sites in which cells of the granulocytic series generally predominated In the other dose groups the regeneration with decreasing dose was more diffusely distributed A persistent predominance of the granulocytic over the erythroid series in the marrow also occurred but started somewhat later with decreasing dose This granulocytosis was intense but temporary in the 0 2 μCi series and decrea ed or disappeared after about 360 days

The bone marrow seemed to play a comparatively small role in the thrombocytopoiesis Even though most of the marrow was destroyed in the highest dose group no peripheral thrombocytopenia developed since sufficient compensatory megakaryocytopoiesis took place in the spleen

A generally diminishing erythropoiesis in the marrow occurred with increasing dose This was however principally taken over by the spleen but was largely of insufficient magnitude as apparent from an increased macrocytosis and a diminished haemoglobin content At the highest dose level and with progressing time this capacity of the spleen gradually decreased in favour of a more accentuated granulocytopoiesis. None of the destructive effects observed

in lymphocytopoiesis could be fully compensated for, and there was as a consequence a discernible shift in the relative proportions of granulocytes and lymphocytes in the peripheral blood

The dose related, persistent and absolute decrease in granulocytes in the peripheral blood principally mirrors the diminished granulocytopoietic capacity, particularly of the bone marrow It is however noteworthy that especially in the two lowest dose groups the granulocytosis, accompanied by a proliferation of myeloid elements, was sometimes of such extent and intensity that it might be identical with the leukaemoid reaction observed in rats by BOEGLER & KRIEGEL (1968), and the so-called myeloproliferative disorders in beagles (GOLDMAN et coll 1969), characterized by a terminal anaemia and a spectrum of disorders varying from myeloid metaplasia to granulocytic leukaemia One of the causes of this reaction may be related to the fact that the granulocytic cell series is more radiation resistent than the erythroid and lymphoid series (1 3)

As regards the mutual relationship between thymus, bone marrow and spleen it is obvious that the involution of the thymus is accelerated and accentuated by an increasing dose, which may be explained on the basis of a dose related scarcity of bone marrow stem cells capable of repopulating the thymus A generally enhanced consumption of thymus lymphocytes with or without destruction by the continuous irradiation from the bones surrounding the thymus may also be factors to consider The spleen seems at least in the higher dose groups to serve as a reserve to compensate for the diminished bone marrow function This interrelationship seems to be of some importance for a better understanding of differences in the leukaemogenesis between ^{90}Sr and fractionated external irradiation Most cases of leukaemia induced by the latter method are of thymic origin (cf JARPLID 1968) in contrast to those induced by ^{90}Sr (8, 13, 18) With fractionated irradiation the entire haematopoietic system is repeatedly and uniformly damaged, and stem cells capable of repopulating the thymus may be acutely lacking (cf JARPLID) The bone marrow is continuously irradiated with ^{90}Sr but the degree of damage and regeneration varies considerably in different parts of the marrow, while other parts of the haematopoietic system initially are not damaged to the same extent as by external irradiation

SUMMARY

The quantitative and qualitative effects of various doses of ^{90}Sr on the haematopoietic system in four groups of CBA male mice and in controls are described

ZUSAMMENFASSUNG

Der quantitative und qualitative Effekt von verschiedenen Dosen von ^{90}Sr an vier Gruppen von mannlichen CBA Mausen und an Kontrolltieren werden beschrieben

RÉSUMÉ

L auteur decrit les effets quantitatifs et qualitatifs de diverses doses de ^{90}Sr sur le systeme hématopoietique dans quatre groupes de souris CBA males ainsi que sur des temoins

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FROM THE RADIATION CENTER, THE UNIVERSITY OF LOUISVILLE, KENTUCKY, U S A

ELECTRON BEAM DEPTH DOSE DISTRIBUTION EVALUATION

by

A JACOBSON TH E BANKS M A ACKERMAN H E BRIZEL and R M SCOTT

The betatron beam therapy program was initiated at the Radiation Center of the University of Louisville when it was opened in 1964 Since that time our dosimetry rationale has placed more reliance on actual measured percent depth dose data than with precise beam energy determinations used in conjunction with published depth dose data SCHULZ (1967) has advanced this viewpoint at the recent conference on high energy radiation therapy dosimetry in New York

Toward this end we have pursued film dosimetry as our principal source of information The known problems of film dosimetry were acknowledged and accepted in the absence of more feasible dosimetry techniques Part of these problems were the tedious and possibly inaccurate manual densitometry of test films There have been several successful approaches to reducing these objections (e g pantographic procedures and use of computers) We are using a commercially available film dosimetry system that is an improved version of the prototype developed by BOGARDUS et coll (1965)

Before giving a brief description of this system the film phantom arrangement will be discussed The loaded phantom positioned for an exposure parallel to the

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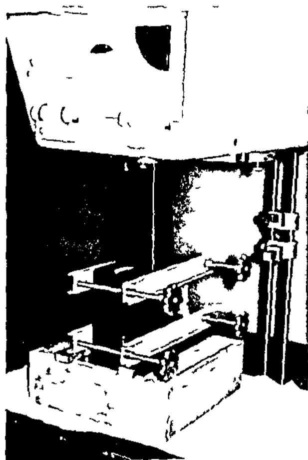


Fig 1 Phantom set up for betatron electron beam exposures Ready pack industrial film is sandwiched in unit density rubber

electron beam is shown in Fig 1 The phantom consists of ready pack industrial film sandwiched between unit density' rubber (Temex) slabs, which are in turn sandwiched in polystyrene slabs Temex rubber was developed in England, as described by STACY et coll (1961) Its density is slightly less than than of polystyrene (Temex $\simeq$ 1 02 versus polystyrene = 1 05) The principal virtue of this approximately unit density rubber is that it forms intimate contact with the test film This is an important consideration in high energy electron film dosimetry because air occlusions result in differential electron scatter, with consequent greater local darkening of the film

Use of light tight, pre wrapped industrial film avoids the cumbersomeness of loading the phantom with bare film in a darkened room In addition to this simplification, we feel there is considerable benefit from more accurately loaded film phantoms than are possible when working in a darkened room For example, in those circumstances where dosimetry is of interest in the electron build up region near the phantom surface it is imperative that the test film be properly located at that margin and clamped securely in the phantom We have found

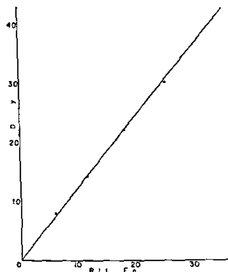


Fig 2 Characteristic curve for industrial type M film exposed to 24 MeV electrons

that the ready pack film gives results in this phantom similar to that of bare film The experimentally determined characteristic curve for the industrial film used in our dosimetry is presented in Fig 2 This particular data is for 24 MeV electrons and we have obtained linear curves for 14 MeV electrons also

Industrial film exposure insensitivity is actually advantageous in this work since there is lesser involvement with background densities The excellent detail property of this type of film makes it ideal for quantification in the densitometry system we use

The film dosimetry system employed for the work described in this paper is manufactured by the Artronix Instrument Company St Louis In Fig 3 is



Fig 3 Film dosimetry system consisting of output writer (with test film) densitometer and printing percent digital voltmeter

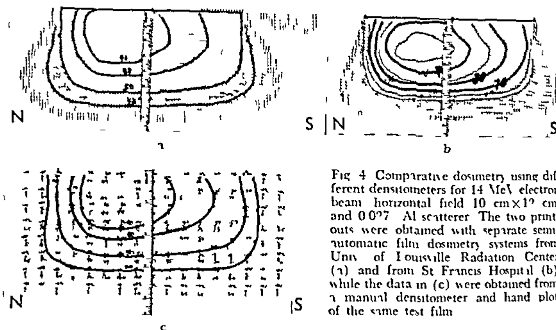


Fig 4 Comparative dosimetry using different densitometers for 14 MeV electron beam horizontal field 10 cm × 12 cm and 0.027 Al scatterer The two print outs were obtained with separate semi automatic film dosimetry systems from Univ of Louisville Radiation Center (a) and from St Francis Hospital (b) while the data in (c) were obtained from a manual densitometer and hand plot of the same test film

shown a test film positioned in the output writer carriage containing a photocell assembly The film is moved past the photocell in a predetermined pattern Thus, the typewriter provides mechanical scanning, as well as a digital print out in 5 % gradations The variable density of the film would result in a variable output current of the PMT However in this system the output current is maintained constant by a feedback method which changes the applied voltage to the PMT thereby providing a logarithmic output to correspond to the film density function This output signal is processed and observed on the densitometer optical density scale A portion of this signal is fed to the input of the printing percent digital voltmeter, where digital display is available

The densitometer range is from zero to four density units and there are three range scales on the densitometer The maximum density of the test film is located by manual movement of the output writer containing the film and simultaneous observation of the optical density on the densitometer It is important to carefully select the reference point, and the zero point as well We have substituted ten turn potentiometers to facilitate selection of these cross over points Periodic checks on the densitometer are made with a calibration wedge, and over all performance of the system is monitored by re measuring of test films of known precision

Comparative data print outs from three film dosimetry units are shown in Fig 4 A 14 MeV electron beam film was densitometered on our Artronix unit, on another such unit at St Francis Hospital in Indianapolis, Indiana, and manually densitometered on our Welch densitometer Qualitative and quantitative

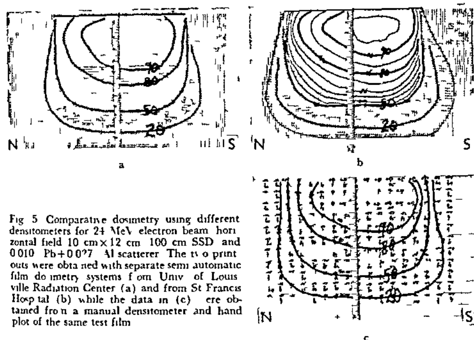


Fig 5 Comparative dosimetry using different densitometers for 24 MeV electron beam horizontal field 10 cm × 12 cm 100 cm SSD and 0 010 Pb + 0 027 Al scatterer The two print outs were obtained with separate semi automatic film dosimetry systems from Univ of Louisville Radiation Center (a) and from St Francis Hospital (b) while the data in (c) were obtained from a manual densitometer and hand plot of the same test film

agreement is quite apparent Equally good comparative data was obtained for a 24 MeV electron beam film densitometered on the same three systems (Fig 5)

Our semi automatic film dosimetry system was used to select the optimum beam flattening material for our electron beam For clinical use it is desired to have the electron beam symmetrical in all planes properly flattened with minimal bremsstrahlung contamination and without excessive loss of beam output Toward this objective lead aluminum and lead aluminum combinations have been used Their location relative to the emergent electron beam is also critical because of their effect on resultant dose distribution We have examined four scatterer arrangements available to us on our machine 0 027 Al *only* 0 010 Pb *only* 0 010 Pb + 0 027 Al and 0 020 Pb *only* All of these scatterers are positioned less than one centimeter from the exit window of the donut and ahead of the parallel plate ionization chamber monitor

The field distributions in the plane perpendicular to the electron beam for all four of these scatterers at 24 MeV are presented in Fig 6 These distributions are in a plane 4 cm below the unit density rubber surface It is seen that only the lightly scattered arrangement (0 027 Al *only*) fails to provide adequate flattening at this energy Incidentally we have found that film dosimetry in the

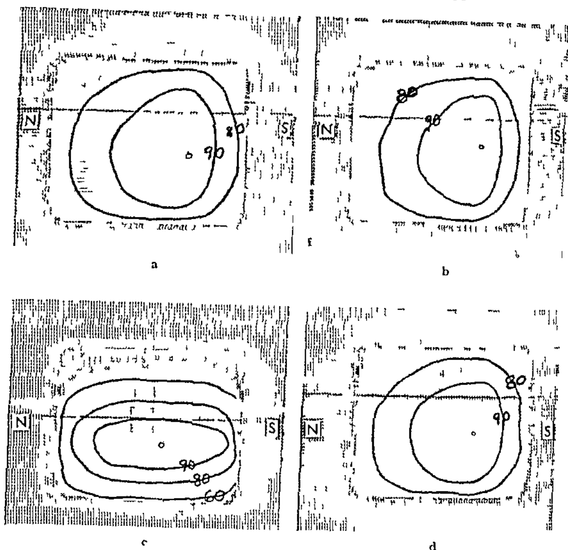
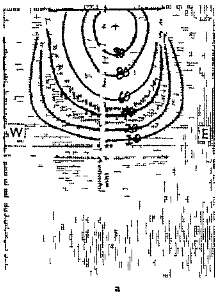
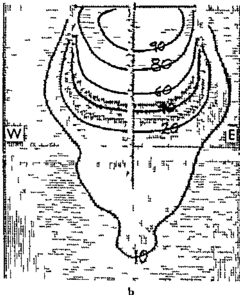


Fig 6 Cross sectional depth dose data (4 cm depth) for four different beam scatterer arrangements and 24 MeV electron beams 10 cm × 12 cm fields 100 cm SSD 0 027 Al *only* (a) 0 010" Pb *only* (b) 0 010 Pb + 0 027 Al (c) and 0 020 Pb *only* (d)

plane shown in this figure, is invaluable in treatment planning For example, the 80 % depth dose line can be clearly visualized in another plane so the clinician may be assured that the tumor mass will be within this isodose boundary The next three diagrams (Fig 7) show central axis dose distributions perpendicular to the plane of the donut (we rountinely take phantom films in the plane parallel to the donut also) It will be seen that practically no bremsstrahlung is evident for the 0 027" Al *only* scatterer But as we have just seen, insufficient beam flattening is provided at 24 MeV The 0 010' Pb *only* and 0 010" Pb + 0 027" Al scatterers resulted in similar amounts of bremsstrahlung as the 0 027" Al *only*



a



b

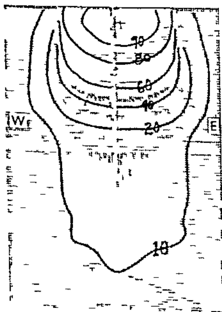


Fig 7 Depth dose data of three different beam scatterer arrangements 0 027 Al *only* (a) 0 010 Pb+0 027 Al (b) and 0 020 Pb *only* (c) These data were obtained from test films exposed in the plane parallel to the betatron donut

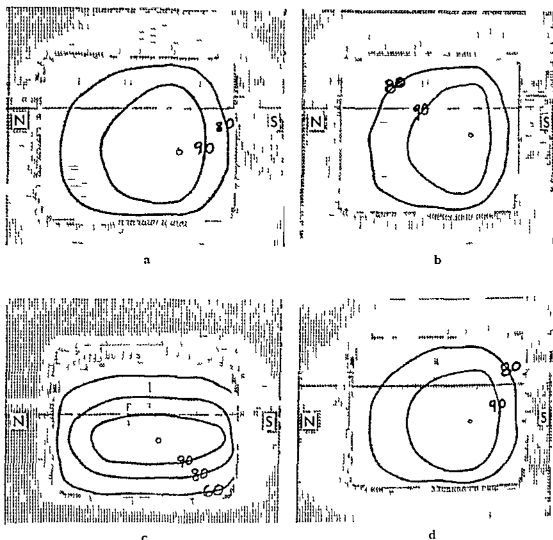


Fig 6 Cross sectional depth dose data (4 cm depth) for four different beam scatterer arrangements and 24 MeV electron beams 10 cm × 12 cm fields 100 cm SSD 0.027" Al *only* (a) 0.010" Pb *only* (b) 0.010" Pb + 0.027" Al (c) and 0 0 0 Pb *only* (d)

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response per rad for electrons and photons was the same within the experimental error of a few percent in the energy range 1—16 MeV

The comparative beam outputs for the four scatterer arrangements are recorded in the Table Sec /Click and R/Click refer to beam monitor counts or 'clicks In our routine calibration method the beam monitor counts are calibrated against a Victoreen ionization chamber in an equilibrium block Thus for the 14 MeV electrons scattered by 0 010′ Pb + 0 027″ Al each beam monitor click representents 6 2 R and the frequency of these clicks gives an estimate of the exposure It will be seen that our election of the single scatterer (0 010′ Pb + 0 027′ Al) is made at the cost of considerable exposure However the avoidance of technician error in improper scatterer selection compensates for this

Sub equent donut replacements may necessitate another electron beam flattening method With the system described here this determination will be simplified greatly over the previous methods available to us

SUMMARY

A recently improved commercially available film densitometry system enabling convenient selection of depth dose distributions is described Suitably obtained radiographic films of test exposures in unit density phantoms are densitometered and automatic digital display of the scanned film is produced in about 15 minutes Examples are shown in which the undesirable bremsstrahlung component of the electron beam is quantified sufficiently for selection of optimum beam flattening arrangements

ZUSAMMENFASSUNG

Ein kurzlich verbessertes kommerziell erhaltliches Film Densitometer System das eine bequeme Selektion der Tiefen Dosis Verteilung ermoglicht wird beschrieben Geeignet exponierte Filme von Test Bestrahlungen in Phantomen von Standard Dichte werden in dem Densitometer gelesen und eine automatisch digitale Darstellung des untersuchten Films kann in etwa 15 Minuten erhalten werden Es werden Beispiele gegeben bei denen die unerwunschte Bremsstrahlungs-Komponente des Elektronenstrahls quantitativ genugend erfasst wird um eine Selektion optimaler Strahlen Abflachung zu ermoglichen

RÉSUMÉ

Les auteurs decrivent un systeme de densitometrie par film recemment perfectionne et commercialise qui permet une sélection convenable de distribution de dose en profondeur Des films convenablement radiographies soumis à des expositions de test dans des fantomes de densite unite ont été lus au densitometre on obtient en 15 minutes environ une representation digitale automatique du film exploré Les auteurs donnent des exemples dans lesquels la composante de bremsstrahlung indesirable du faisceau d electrons est suffisamment quantifiee pour qu on puisse choisir les arrangements qui donnent le meilleur aplatissement du faisceau

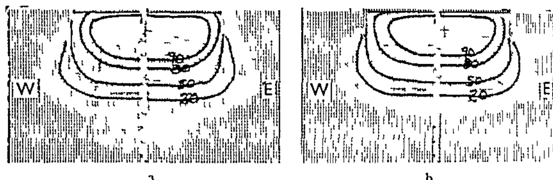


Fig. 8. Depth dose data with 14 MeV electron beam using 0.010" Pb *only* scatterer (a) and 0.010" Pb+0.027" Al scatterer (b). Increased bremsstrahlung component below 20% isodose for the 0.010" Pb *only* scatterer.

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Fig. 8 shows our basis for selection of the combination scatterer. These distributions are taken at 14 MeV and it is seen that there is some additional bremsstrahlung present with the 0.010" Pb *only* scatterer.

Comparative quantification of the bremsstrahlung component in the high energy electron film introduces no serious error. DUDLEY (1966) has discussed this question in the new edition of Radiation dosimetry. It was found that

Table

Comparison of electron beam outputs for various scatterer arrangements using a field size of 10 cm × 12 cm and 100 cm SSD

	Scatterer	Sec./Click	R/Click
14 MeV	0.027" Al	~2.0	12.4
	0.010" Pb	~4.5	6.3
	0.010" Pb+0.027" Al	~4.7	6.2
24 MeV	0.0[illegible]0" Pb	~5.3	5.7
	0.027" Al	~4.9	14.0
	0.010" Pb	~6.0	7.6
	0.010" Pb+0.027" Al	~6.9	7.6
	0.020" Pb	~8.9	7.0

response per rad for electrons and photons was the same within the experimental error of a few percent in the energy range 1—16 MeV

The comparative beam outputs for the four scatterer arrangements are recorded in the Table Sec/Click and R/Click refer to beam monitor counts or clicks In our routine calibration method the beam monitor counts are calibrated against a Victoreen ionization chamber in an equilibrium block Thus, for the 14 MeV electrons scattered by 0 010′ Pb + 0 027″ Al each beam monitor click represents 6 2 R and the frequency of these clicks gives an estimate of the exposure It will be seen that our election of the single scatterer (0 010′ Pb — 0 027 Al) is made at the cost of considerable exposure However the avoidance of technician error in improper scatterer election compensates for this.

Subsequent donut replacements may necessitate another electron beam flattening method With the system described here this determination will be simplified greatly over the previous methods available to us

SUMMARY

A recently improved commercially available film densitometry system enabling convenient selection of depth dose distributions is described Suitably obtained radiographic films of test exposures in unit density phantoms are densitometered and automatic digital display of the scanned film is produced in about 15 minutes Examples are shown in which the undesirable bremsstrahlung component of the electron beam is quantified sufficiently for selection of optimum beam flattening arrangements

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Ein kurzlich verbessertes kommerziell erhaltliches Film Densitometer System das eine bequeme Selektion der Tiefen Dosis-Verteilung ermoglicht, wird beschrieben. Geeignet exponierte Filme von Test Bestrahlungen in Phantomen von Standard Dichte werden in dem Densitometer gelesen und eine automatisch digitale Darstellung des untersuchten Films kann in etwa 15 Minuten erhalten werden. Es werden Beispiele gegeben bei denen die unerwunschte Bremsstrahlungs-Komponente des Elektronenstrahls quantitativ genugend erfasst wird um eine Selektion optimaler Strahlen Abflachung zu ermoglichen

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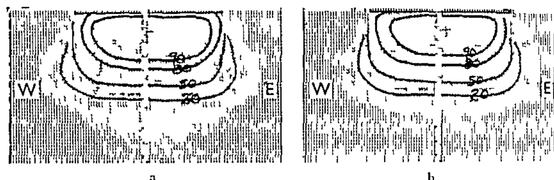


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	0 010 Pb	~4 5	6 3
	0 010 Pb+0 027 Al	~4 7	6 2
	0 020 Pb	~5 3	5 7
24 MeV	0 027 Al	~4 9	14 0
	0 010 Pb	~6 0	7 6
	0 010 Pb+0 027 Al	~6 9	7 6
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FROM THE DEPARTMENT OF RADIOLOGICAL SCIENCES, THE JOHNS HOPKINS SCHOOL OF HYGIENE AND PUBLIC HEALTH BALTIMORE, MARYLAND, U S A

KILOVOLTAGE CALIBRATION OF DIAGNOSTIC ROENTGEN RAY GENERATORS

by

JAGDISH P BHATNAGAR and GOPALA U V RAO

With the development of modern shock proof roentgen ray tubes several indirect methods not calling for direct access to the high voltage leads have been proposed for the kilovoltage calibration of roentgen ray machines Of these the one that uses a crystal monochromator is the most accurate However, in view of its complexity and unsuitableness for routine measurements a method that is based on the measurement of the intensity of K characteristic radiation resulting from the interaction of a roentgen beam with an interposed radiator is often preferred

GREENING (1955) first proposed a method based on this principle In his method the radiator is placed at an angle of 45° to the axis of the incident beam (Fig 1) An ionization chamber placed at right angles to the roentgen beam measures the 90 secondary radiation exposure rate (*Rs*) from the radiator Another chamber is placed in the transmitted beam to measure the transmitted beam exposure rate (*Rt*) Both the transmitted and secondary radiation exposure rates are measured for variou kV settings of the roentgen ray machine The ratio of *Rt*/*Rs* for each kV setting is plotted against the kV etting GREENING established that in the resulting inverted V shaped curve the peak corresponded to the

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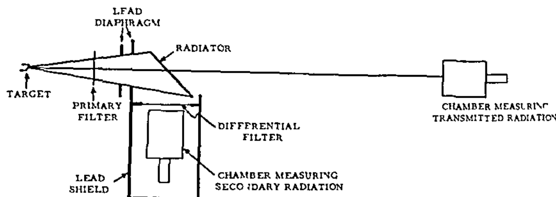


Fig 1 Experimental arrangement for the kilovoltage calibration of a roentgen unit

actual keV of the K-edge of the radiator By the choice of several radiators, the calibration can be carried out up to a maximum kilovoltage of 115 6 kV corresponding to the K absorption edge of uranium A differential filter placed in front of the ionization chamber that measures *Rs* serves to improve the accuracy of the method

A few years ago, Bloch & Hale (1966) suggested another method to determine the kV settings corresponding to the K absorption edges of the radiators In this method, a thin window NaI (Tl) scintillation spectrometer is used instead of an ionization chamber to measure the 90° secondary radiation The pulse height analyzer of the spectrometer is set with the center of the window at the K characteristic radiation energy of the radiator The number of counts collected over a given time is plotted as a function of the roentgen tube potential The resulting plot shows a linear portion which is extended to intercept the tube potential axis (x axis) The intercept in this method gives the potential of the roentgen tube equivalent to the K absorption edge of the radiator

Recently, Davison & Reekie (1968) have suggested a method of kV measurement in which only the 90° secondary radiation (*Rs*) from the radiator is measured by an ionization chamber The incident primary beam on the radiator is heavily filtered, and the differential filter of Greening is replaced by a thin aluminum filter *Rs* is then plotted against the kV meter readings The kV meter reading at which the K characteristic radiation starts contributing to the 90° secondary radiation is known as the 'breakaway' point The success of the method therefore depends on the accuracy with which this 'breakaway' point can be determined

In an attempt to calibrate the kV-meter of a roentgen ray machine, using the methods of Greening (1955), Bloch & Hale (1966), and Davison & Reekie (1968) we found a number of uncertainties associated with each of these meth

Table

Intercomparison of kV-calibration methods

Radiator and K absorption edge energy	Differential filter	Thickness of radiator in mm	Kilovoltage at the K edge position as detected by the method of		
			GREENING	BLOCH & HALE	BHATNAGAR & RAO
Ta (67.4 keV)	0.15 mm Pb	0.5	71	71.5	69.5
		1.0	74		69.5
Au (80.7 keV)	0.15 mm Pb	0.075	no peak obtained		82.5
		0.125	85		82.5
		0.25	87		82.5
		0.375	87.5		82.5
		0.50	88	84	82.5
Pb (88.0 keV)	0.15 mm Pb	0.125	92		91.0
		0.25	95		91.0
		0.375	96		91.0
		0.50	96.5		91.0
		0.625	97		91.0
		0.75	97.5		91.0
		1.00	98	93	91.0
U (115.6 keV)	0.26 mm U	0.264	121		121.5
		0.528	124		121.5

ods In this paper we propose to discuss these uncertainties and suggest a method which gives accurate results to within ± 1 % up to 115 kV Theoretical justification in support of the proposed method will also be presented

The roentgen rays used to obtain the data reported in this paper were produced by a Siemens constant potential generator operated in the range of 60—120 kV The experimental set up used for the study is shown in Fig 1 The radiation detectors used in most of our experiments for measuring the transmitted and the 90° secondary radiation exposures were standard Victoreen R chambers coupled to a Townsend Balance circuit The radiators used were Ta Au Pb, and U Their thicknesses and K absorption edge energies are given in the Table in which the differential filters used and their thicknesses are also given

In order to repeat the BLOCH & HALE (1966) method a thin window

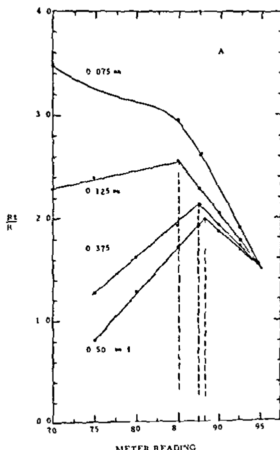


Fig 2 Results obtained with the GREENING (1955) method for various thicknesses of a gold radiator There is no peak in evidence at a thickness of 0 075 mm and for higher thicknesses the peak goes on shifting towards higher kV meter readings

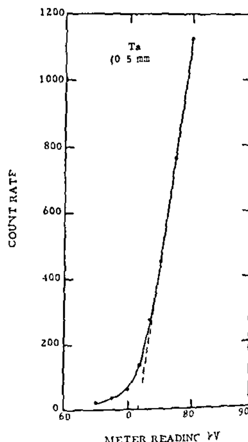


Fig 3 Results obtained with the BLOCH & HALE (1966) method for Ta radiator (0 5 mm thick) Intercept of dotted line with kV axis is expected to yield the K absorption edge of the radiator but in practice this intercept occurs at a value considerably higher than the K absorption edge

cm Al) NaI (Tl) scintillation spectrometer with a single channel analyzer was used The scintillation spectrometer was calibrated using standard gamma sources of ^{109}Cd (22 keV), ^{133}Ba (32 keV) and ^{170}Tm (84 keV) A window width of 2 keV was used

Results

The Greening method The results obtained using the GREENING (1955) method for various thicknesses of a gold radiator are given in Fig 2 For convenience, the values of Rt/Rs are normalized at a meter reading of 95 kV This figure clearly illustrates how the position of the inverted V peak depends considerably

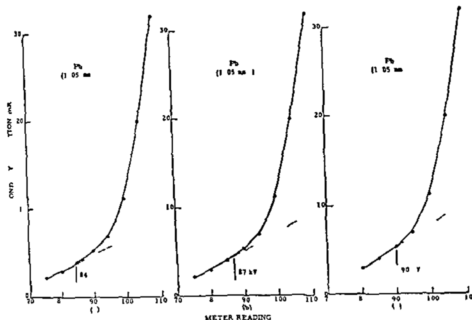


Fig 4 *Results* obtained with the DAVISON & REEKIE (196?) method for a lead radiator Three different straight lines drawn through the same set of data yield three different breakaway points at 84 87 and 90 kV The 75 kV data point in (c) is deleted purposely to demonstrate that the position of the 'breakaway point depends on the kV setting at which the measurements are started

on the thickness of the radiator used The peak is obtained at different values of kV for different radiator thicknesses Furthermore at a thickness of 0 075 *mm or* less there does not exist a peak at all The data obtained with all the radiators used in our study are summarized in the Table p 557 It is seen that in all cases the position of the peak keeps on shifting further and further away towards the high voltage side with increase in thickness of radiator

This shift of peak position seems to occur because of the variation of *Rt* with increase in radiator thickness Closer investigation showed that while *Rs* initially increases and soon reaches a saturation value at some critical thickness *Rt* on the other hand decreases steadily with radiator thickness (In the case of gold and lead the critical thickness was found to be 0 2 mm and in the case of tantalum and uranium the minimum thicknesses available to us were not small enough to detect this variation) Moreover the shape of *Rt* kV curve was observed to change significantly with radiator thickness Therefore when the ratio *Rt*/*Rs* is plotted against kV for different radiator thicknesses one observes a shift in peak position mainly due to the variation in *Rt*

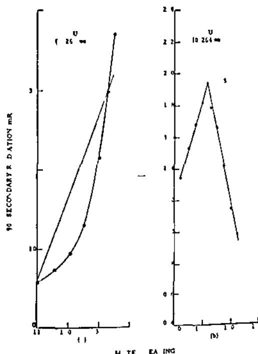


Fig 5 Diagram illustrating the procedure proposed for an accurate determination of the K absorption edge An arbitrary straight line is drawn on the Rs kV curve in such a manner as to intersect it at points as far separated as possible For different kV settings the ratios of the ordinates of points on the straight line to those on the Rs kV curve are plotted as a function of kV setting (b) The K absorption edge is indicated by the peak of the inverted V shaped curve that results

The Bloch & Hale method A typical result obtained with the Bloch & Hale (1966) method for Ta radiator (0 5 mm thick) is recorded in Fig 3 A comparison of results obtained by this method for Ta (0 5 mm thick), Au (0 5 mm thick) and Pb (1 05 mm thick) with the Greening method at these thicknesses is given in the Table It is seen that the values obtained by the Bloch & Hale method are different from those obtained by the Greening method at the thicknesses under consideration Additionally, the former method is open to question (DAVISON & REEKIE 1968) due to the fact that the response of a scintillation detector spreads the monochromatic radiation into a Gaussian type of distribution, and consequently the counts taken from a window set to the characteristic radiation energy of the radiator will not only be due to characteristic radiation but also due to non characteristic scatter from the radiator For these reasons the intercept of the extrapolated portion of the kV count rate curve with the kV axis (Fig 3) is not likely to indicate the correct K absorption edge of the radiator In general, the intercept occurs at a value higher than the K absorption edge of the radiator

The Davison & Reekie method Using the DAVISON & REEKIE (1968) method we experienced considerable difficulty in deciding the points through which a straight line should be drawn to find out the 'breakaway' point It may be seen from Fig 4 that three different straight lines can be drawn through the same

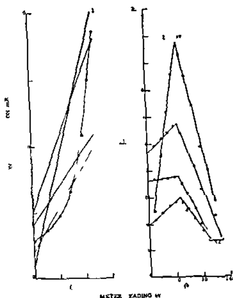


Fig 6 Diagram illustrating that the position of the peak of the inverted V shaped curve is independent of the slope of the arbitrary straight line

et of data giving the breakaway points at 84 87, and 90 kV It is not known which of these three breakaway points is the correct one In Fig 4c the 75 kV data point is deleted purposely to demonstrate that the position of the breakaway point depends on the kV setting at which the measurements are started Similar difficulty was experienced in the case of all other radiators It thus seems that the Davison & Reekie method of finding out the breakaway point for a radiator could largely depend on an individual s personal judgement This difficulty arises from the fact that the 90 secondary radiation is not a linear function of the applied kilovoltage It has a definite curvature and there can be drawn any number of straight lines by observation through points lying on a curve

Proposed modification of Greening s method

The difficulties encountered with the methods discussed above can be overcome by the following procedure

On a plot of the 90 secondary radiation *Rs* (as measured by an ionization chamber) against the kV setting of the machine (Fig 5a) an arbitrary straight line *Sl* is drawn If now for different kV settings the ratios of the ordinates of points on the straight line to those on the *Rs* kV curve are plotted as a function of the kV setting an inverted V shaped peak results (Fig 5b) The position of the peak occurs at the point where the trend of the *Rs* kV curve changes due to

the addition of the K characteristic radiation from the radiator The position of this peak is independent of the slope of the straight line as illustrated in Fig 6 This in fact, is what one would expect, as can be seen from the following

The 90° secondary radiation exposure rate, $Rs(V)$, at any kilovoltage V is given by the equation

$$R_s(V) = I_{coh}(V) + I_{incoh}(V) + I_{ch}(V) \tag{1}$$

where $I_{coh}(V)$ and $I_{incoh}(V)$ are the exposure rate components due to the coherent and incoherent scattering respectively and $I_{ch}(V)$ is the component due to the characteristic radiation from the radiator

At tube voltages less than the K-absorption edge of the radiator, eq (1) simply reduces to

$$R_s(V) = I_{coh}(V) + I_{incoh}(V) \tag{2}$$

Now if $Y = mV + C$ is assumed to be the equation for the arbitrary straight line drawn on the Rs kV curve, it follows that the ratio Q of the ordinate of this straight line to that of the Rs kV curve at any tube voltage V is given by

$$Q = \frac{mV + C}{I_{coh}(V) + I_{incoh}(V)} \qquad \text{when } V < V_k \tag{3}$$

$$Q = \frac{mV + C}{I_{coh}(V) + I_{incoh}(V) + I_{ch}(V)} \qquad \text{when } V > V_k \tag{4}$$

where V_k is the voltage corresponding to the K absorption edge of the radiator

Assuming that the arbitrary straight line is drawn above the Rs kV curve as in Fig 5a and in Fig 6a, it follows from eq (3) that Q is an increasing function of V as long as V is less than V This is because, in this region, the numerator, which is the ordinate of the arbitrary straight line increases much more rapidly than the sum of the terms I_{coh} (V) and I_{incoh} (V) in the denominator However as soon as V becomes greater than V_k Q begins to decrease with V because of the sudden addition of the rapidly increasing term I_{ch} (V) to the denominator (eq 4 and Fig 7) Hence the peak of the inverted V shaped curve of Q versus kV always occurs at the K absorption edge of the radiator regardless of the slope of the arbitrary straight line In practice, for greater accuracy we found that the following criteria should be observed while drawing the arbitrary straight line First of all, care should be taken to draw the straight line sufficiently above the Rs kV curve Secondly, it should be drawn in such a way as to cut the Rs kV curve at two points, one point being on the low kV side where the increase of Rs is slow and the other point on the high kV side where Rs increases rapidly with kV

The Table, p 557, shows the results obtained by this method of kV calibra

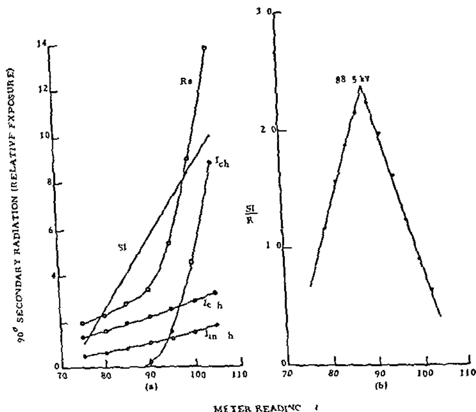


Fig 7 *Theoretical* confirmation of the straight line method of kV calibration In (a) are shown the values of I h I h and I h as a function of tube voltage calculated theoretically for a 2 mm Al primary filter and a 0 15 mm lead differential filter $Rs \approx I_{h} + I_{oh} + I_{ch}$ In (b) the straight line method is applied to *these theoretical* values of *Rs* The peak of the resulting V shaped curve is seen to occur at 88 5 kV while the K absorption edge energy of lead is 88 keV

tion for a number of radiator thicknesses It will be seen from this table that for the entire range of thicknesses used with gold the position of the peak occurred at 82 5 kV Likewise the peak always occurred at 91 0 kV for lead Similarly in the case of tantalum and uranium quite satisfactory results were obtained

The accuracy with which the K absorption edge of a radiator can be determined by the method outlined above can be tested in one of two ways The first one is to make a direct check with a sphere gap An alternative method is to make a theoretical estimate of *Rs* for different values of kV in the case of a typical radiator and then use it to estimate its K absorption edge by the method under test The second method was chosen because of the inaccessibility to the high

the addition of the K-characteristic radiation from the radiator The position of this peak is independent of the slope of the straight line as illustrated in Fig 6 This in fact, is what one would expect, as can be seen from the following

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where V_{ie} is the voltage corresponding to the K absorption edge of the radiator

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The Table, p 557, shows the results obtained by this method of kV calibra

$$I_{coh}(V) = 8\ 789 \int^{V} (V - E) |F/Z|^2 \, {}_E(\mu_{en}/\varrho)_{ir} \exp(-\mu_p l - \mu x)\, dE$$

$$I_{ch}(V) = 1\ 15 \times 10^{21} \int_{V_K}^{V} \frac{(V - E)}{E} \tau \, {}_{E_k}(\mu_{en}/\varrho)_{ir} \exp(-\mu_p l - \mu_k x)\, dE$$

where V	= maximum photon energy present in the primary beam (equal to the applied kV) in keV
E	= photon energy less than V in keV
E'	= energy of the photons scattered at 90 in keV
$d\sigma/d\Omega$	= Klein Nishina differential cross section in cm²/electron per unit solid angle at 90
S	= reduction factor to correct ($d\sigma/d\Omega$) for bound electrons (Also known as incoherent scattering function)
μ μ' μk	= linear attenuation coefficients in cm ¹ of differential filter for photon energy E scattered photon energy E' and K-characteristic radiation energy E_k respectively
x	= thickness in cm of the differential filter
μ_p	= linear attenuation coefficient in cm ¹ of the filter in the primary roentgen beam for photon energy E
l	= Thickness in cm of the filter in primary roentgen beam
${}_E(\mu_{en}/\varrho)_{ir}$ ${}_{E'}(\mu_{en}/\varrho)_{ir}$ ${}_{EK}(\mu_{en}/\varrho)_{ir}$	= mass energy absorption coefficient in cm²/g for air for photon energy E scattered photon energy E' and K-characteristic photon energy E_k
$\lvert F/Z \rvert$	= atomic structure factor per electron of the radiator of atomic member Z
τ	= photoelectric cross-section for the entire atom in cm²/atom
V_K	= K absorption edge energy of the radiator in keV (88 keV for the lead radiator)

Using the appropriate values of $d\sigma/d\Omega$ S μ τ (GRODSTEIN 1957) $(\mu_e/\varrho)_{ir}$ (ICRU 1962) F/Z (NELMS & OPPENHEIM 1955) V_K and E_k (FINE & HANDEE 1955) we estimated the relative values of I_{inch} I_{coh} and I_{ch} by numerical integration The results are shown in Fig 7a in the case of a lead radiator

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The authors wish to thank Mr P N Krishnamoorthy and Mr P Gangadharan for their interest and encouragement in the early phases of this work Thanks are also due to Mrs Laverne Fair for typing the manuscript

SUMMARY

The uncertainties associated with the current methods of kV-calibration of diagnostic roentgen ray generators making use of the K absorption edges of different elements are discussed A method of eliminating these uncertainties is proposed The proposed method is based on a measurement of the 90 secondary radiation (R_s) from an interposed radiator in the primary roentgen beam at different kV settings of the roentgen ray generator

used in these theoretical calculations is given in the appendix. The values of *Rs* calculated in this manner for a lead radiator were plotted as a function of kV (see Fig 7) and the procedure outlined above applied to this theoretical *Rs* kV curve. When this was done, an inverted V shaped curve resulted, with a peak at 88.5 kV. Since the K absorption edge of lead was assumed to be 88 keV in the theoretical calculations, it follows that the value obtained by the straight line method is within 1 % of the true value. It may therfore be expected that a determination of the K absorption edge by this method from an experimentally determined *Rs* kV curve is also equally accurate.

Conclusion

Experimental and theoretical evidence in support of an accurate method for the kilovoltage calibration of diagnostic roentgen ray units, based on a measurement of the secondary radiation alone from an interposed radiator, is presented. The method is essentially a modification of Greening's original method. While in Greening's method, the ratio *Rt/Rs* is plotted against kV, in our case the measurement of *Rt* is altogether eliminated and the numerator is replaced by a linear function of kV. The transmitted radiation exposure rate that Greening uses in the numerator is known to be a power function of the voltage and varies with the thickness of the radiator. The calibration is therefore subject to unpredictable errors depending on the thickness of the radiator. In our method since the *Rt* kV curve is replaced by a simple straight line, these uncertainties no longer exist. The modified procedure suggested here is especially suited for day to day field work and is worthy of consideration for being developed into a single package kilovoltage calibration unit.

Appendix

Theoretical calculation of secondary radiation from a radiator

Consider a lead radiator of thickness *dt* placed in a roentgen beam. Let the secondary radiation from it be filtered by a differential lead filter of thickness *l* cm. The resultant 90 secondary radiation exposure *Rs* (V) at any kilovoltage *v* is given by the equation

$$R_s\ (V) = I_{coh}\ (V) + I_{incoh}\ (V) + I_{ch}\ (V)$$

where I_{coh}(V) and I_{incoh}(V) are the components due to coherent and *incoherent scattering* respectively and I_{ch}(V) is the component due to characteristic radiation.

Making use of the well known equation of KRAMER (1923) for the spectrum of the primary roentgen beam *incident on the radiator* it can be shown that I_{coh}, I_{incoh} and I_{ch} for a lead radiator are given by the following expressions

$$I_{incoh}\ (V) = 0.275 \times 10^{25} \int^{1} \frac{1 - E}{E} \left(\frac{d\sigma}{d\Omega} \right) S\ E\ \ F\ (\mu_{en}/\rho)_{air} \times \frac{510 + E}{510 - E} \exp\ (-\mu_p l - \mu\ x)\ dE$$

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TECHNETIUM 99m SULFIDE COLLOID PREPARATION FOR SCINTIGRAPHY OF THE RETICULOENDOTHELIAL SYSTEM

by

R BERTIL R PERSSON and YNGVE NAVERSTEN

The technetium $^{99}Tc^{m}$ was first used by HARPER (1964) in a fat emulsion and later as a sulfide colloid for liver scintigraphy In 1966 HARPER et coll prepared the sulfide colloid by introducing H S gas into an acidified pertechnetate solution in the presence of gelatin This method was somewhat complicated for routine use and the yield was a mixture of colloidal and soluble technetium Simplified methods for the preparation of $^{99}Tc^{m}$ sulfide colloids have been developed by PATTON et coll (1966) STERN et coll (1966) and LARSSON & NELP (1966) The method described by PATTON et coll employed perrhenate ($\simeq$ 0 002 M) as the carrier of pertechnate and formed the sulfide colloid in the presence of thiosulfate ($\simeq$ 0 010 M) at a low pH value ($\simeq$ 1) and heated to about 100 C The experimental conditions for this method have been investigated by LARSSON & NELP and are well known STERN et coll found that if perrhenate was excluded and the pH value readjusted beyond 6 5 the dissociation of the colloid would take place

Different agents have been used to stabilize the colloid e g gelatin by HARPER

Submitted for publication 13 November 1969

ZUSAMMENFASSUNG

Die mit den gangigen Methoden der kV Kalibrierung von diagnostischen Rontgenapparaten verbundenen Unsicherheiten bei der Anwendung der K Absorptionskanten verschiedener Elemente werden diskutiert Eine Methode diese Unsicherheiten zu eliminieren wird vorgeschlagen *Die vorgeschlagene Methode grundet sich auf eine Messung der 90°* Sekundar Strahlung (*Rs*) von einem in den primaren Rontgen Strahl bei verschiedener kV Einstellung des Rontgenapparates gebrachten Strahlers

RÉSUMÉ

Les auteurs etudient les incertitudes que comportent les methodes habituelles d etalonnage de la tension des générateurs de radio diagnostic par l utilisation des pics de l absorption K de differents elements Ils proposent une methode qui elimine ces incertitudes elle est basee sur la *mesure du rayonnement secondaire (Rt) émis a 90* *par une source secon* daire interposee dans le faisceau du rayon primaire pour differentes tensions du génerateur de rayon de roentgen

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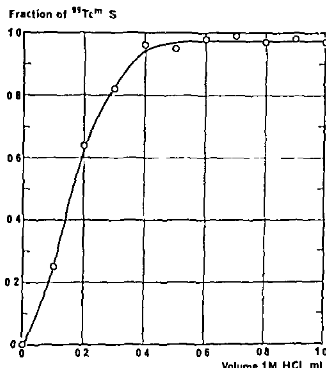


Fig 1 The fraction of ^{99}Tcm retained as sulfide colloid after filtration through a 0 45 μm millipore (HA) filter when varying volumes of 1 M HCl were added to the stock solution before heating for 3 min in a boiling water bath

et coll and by PATTON et coll , dextran by LARSSON & NELI and by DWORKIN et coll (1967), carboxymethyl cellulose (CMC) by STERN et coll and polyvinylpyrrolidone (PVP) by GARZON et coll (1966), SZYMENDERA et coll (1969) and EGE & RICHARDS (1969) A survey of 29 institutions in the U S A conducted by SMITH et coll (1967) revealed anaphylactic and anaphylactoid reactions in 0 9 % of patients given ^{99}Tcm sulfide colloid stabilized with dextran These results were confirmed by the finding of BAILEY et coll (1967) that high molecular dextran induces anaphylaxis acting as a non specific histamine releaser in patients pre sensitized to native dextran With gelatin as a stabilizing agent, however, fewer than 0 1 % reactions were reported by SMITH et coll and these could not be definitely associated with the colloid WEBBER et coll (1969) eliminated the risk related to the use of dextran by preparing the colloid without a stabilizer They obtained however a suspension, not a colloid, and as this precipitates it was necessary to resuspend it by shaking before administration They are now looking for a nonallergenic stabilizer because the amount of soluble technetium increases when left standing several hours after its preparation The effect on the colloid by omitting a stabilizer has also been studied by LOPEZ &

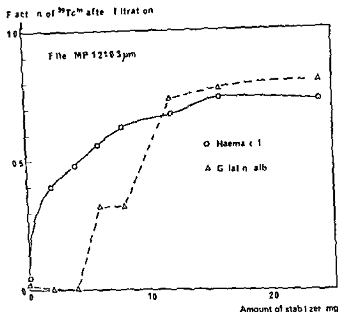


Fig 7 The ratios between the ^{99}Tc^{m} radioactivity concentration (mCi/ml) after and prior to filtration through a *millipore filter* 1 2 μm with various amounts of Haemaccel or Gelatin Alba as stabilizing agent

French (1969) they too observed some aggregation of the colloid particles in vitro but no significant uptake in the lungs was evident in the scintigrams By reducing the amount of sodium thiosulfate to one tenth they got no measurable precipitation but the amount of free ^{99}Tc^{m} increased from 2 % directly after the preparation up to 4 2 % five hours later

We wanted to further improve the method described by Patton et coll and by Larsson & Nelp and we decided to use gelatin as a stabilizing agent Two pharmaceutical materials were available Gelatin Alba (USA) and Haemaccel The latter is a commercially available plasma expander free from pyrogenic and antigenic reactions as reported by Scherer & Wunsch (1963) and by Hassig (1966)

The following chemicals and reagents were used in the preparation of the ^{99}Tc^{m} ulfide colloid

Chemicals

Sodium thiosulfate pro analysi (Merck Germany) Na S O_3 5H O Mol wt 248 18

Technetium 99m generator (The Radiochemical Centre Amersham England) code MCC 2

Sodium phosphate pro analysi (Merck Germany) Na HPO 7H O M

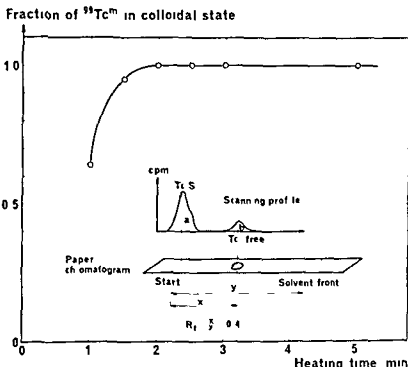


Fig 3 Fraction of $^{99}Tc^{m}$ activity incorporated in colloidal sulfide particles after heating in boiling water at various time intervals

Potassium perrhenate (Hopkin & Williams Ltd England) $KReO_4$ Mol wt 289 32

Hydrochloric acid pro analysi (Riedel—DeHaen AG Germany) HCl Mol wt 36 47 37 %

Haemaccel (Farbwerke Hoechst AG Germany) gelatin polymerizate 3 5 % solution ave Mol wt 35 000

Gelatin Alba (USA) gelatin powder for pharmaceutical use

Sodium chloride (ACO Sweden) NaCl 0 9 % for infusion

Reagents

Stock solution 0 600 g of $Na_2S_2O_3$ $5H_2O$ 0 125 g of $KReO_4$ 18 ml Haemaccel 3 5 % sterile water added to 100 ml

A sterile kit for 10 preparations of Tc S consists of 10 stock solutions each 2 ml in 20 ml bottles 1 bottle 1 M hydrochloric acid 10 ml 1 bottle 0 2 M Na_2HPO_4 solution 50 ml

Procedure The $^{99}Tc^{m}$ eluate from the generator was obtained in 5 ml fractions of 0 9 % NaCl in multidose bottles and sterilized at 120° C for 20 minutes One fraction was transferred to a 20 ml multidose bottle containing 2 ml of the stock solution The colloid was formed by heating in a boiling water bath for 3 0 minutes After cooling, the acidity was adjusted to pH = 6 4 by adding 4 0 ml of 0 2 M Na_2HPO_4 The Tc S colloid was then ready for clinical use

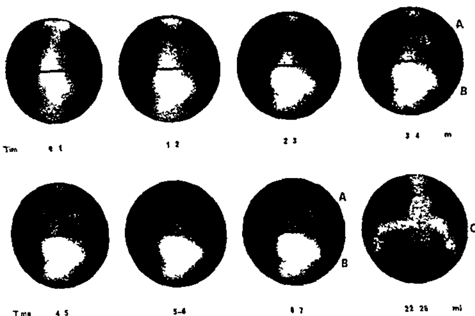


Fig. 4. Gamma camera photographs obtained at different times after intravenous administration of Tc-S colloid in the ear vein of a rabbit in supine position. The upper (cranial) half of the view is called field A in the text and the lower (caudal) half field B. The picture in the lower right corner is the pelvis and backbones in the same position, exposure time 4 minutes, termed field C in the text.

The normal activities to be used for liver and bone marrow scintigraphy are 2—3 mCi and 5—10 mCi, respectively.

This procedure differs from that described by PATTON et coll. and by LARSSON & NELP by the use of only 0.5 ml 1 M HCl instead of 1.0 ml. It was thus possible to neutralize the solution with only 4 ml 0.2 M Na_2HPO_4 in place of 3 ml of the concentrated buffer mixture suggested by these authors, and this resulted in an easily prepared solution and an almost isotonic final product. Furthermore, the use of Haemaccel as stabilizing agent excludes the risk of pyrogenic and antigenic reactions associated with the stabilizer.

In vitro tests of the colloid

Filtration tests. The efficiency of the colloid formation was investigated with different amounts of 1 M HCl added to the stock solution before heating in boiling water for 3 minutes. After pH adjustment, 500 μl were taken as a reference and the rest was passed through a 0.45 μm millipore (HA) filter. The

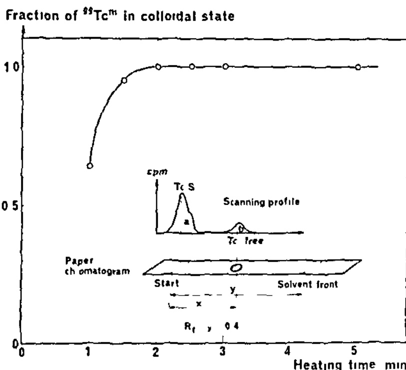


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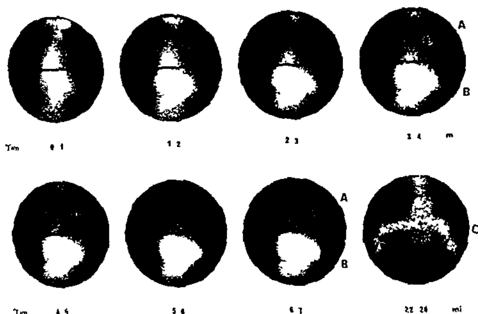


Fig 4 Gamma camera photographs obtained at different times after intravenous administration of Tc S colloid in the ear vein of a rabbit in supine position The upper (cranial) half of the view is called field A in the text and the lower (caudal) half field B The picture in the lower right corner is the pelvis and backbones in the same position exposure time 4 minutes termed field C in the text

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In vitro tests of the colloid

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Table 1

Ratio between number of counts in field A (cranial) and field B (caudal) with different amounts of Gelatin Alba or Haemaccel

Amount mg	Gelatin Alba counts in A/counts in B	Haemaccel counts in A/counts in B
0	0 056	0 056
12	0 165	0 074
16	0 092	0 074
24	0 074	0 076

Table 2

Ratios between number of counts in lung field A and liver field B and ratios between number of counts in pelvic field C and liver field B at varying heating times during the preparation of the Tc S colloid

Heating time (100 C) min	Ratio counts in A/counts in B	Ratio counts in C/counts in B
2	0 072	0 071
3	0 089	0 083
5	0 055	0 087
7	0 100	0 103
10	0 069	0 075
Mean ± S D	0 077 ± 0 020	0 084 ± 0 014

fraction of $^{99}Tc^{m}$ S stopped by the filter, as a function of the volume 1 M HCl added, is given in Fig 1

When more than 0 4 ml 1 M HCl was added, nearly all the colloidal particles were stopped by the filter, but if less 1 M HCl was added, the $^{99}Tc^{m}$ was not fixed quantitatively in the colloidal state To obtain a moderate volume of the preparation, we decided to use 0 5 ml 1 M HCl

The colloid size was also studied with varying amounts of stabilizing agent It was prepared as described above except that the amounts of Haemaccel and Gelatin Alba in the stock solution were varied The colloid one hour after the preparation was passed through a 1 2 μm millipore (RA) filter Aliquots were taken and the results are given in Fig 2 as the ratio of $^{99}Tc^{m}$ activity concentration after and prior to filtration This investigation indicated that a small amount of Haemaccel is sufficient to produce a stabilization effect on the colloid As more than 12 mg is without further effect, this amount was employed Small quantities of Gelatin Alba failed to have as good a stabilizing effect as Haemaccel

Table 3

Ratio between number of counts in field A (cranial) and field B (caudal) with varying time intervals between the preparation and administration of the Tc-S colloid

Time interval between preparation and administration	Counts in A/counts in B
15 minutes	0 047
4 hours 30 minutes	0 041
7 hours 15 minutes	0 055

but in excess of 12 mg produced about the same result The efficiency of colloid formation as a function of heating time was investigated with paper chromatography A small volume (100 μl) was placed on Whatman paper No 1 the lower part of which was immersed in 85 % methanol solution for 6 hours The paper then was scanned with a slit collimated ϕ 3 $\times$ 2 NaI(Tl) crystal (Fig 3) The results obtained after varying the heating time are given in the figure as the fraction a/(a + b) of ^{99}Tc^{m} activity incorporated in the colloidal particles A plateau was obtained after 2 minutes heating but 3 0 minutes were adopted for routine use

Colloidal particle size One drop of the colloid was spread on a glass plate and after drying was viewed in a light microscope through immersion oil The mean size of the colloidal particles was estimated to be 0 6 $\pm$ 0 2 μm Another drop was put on a copper grid and dried for 30 minutes before it was viewed in a electron microscope The mean size was estimated from photographs (16 000 $\times$ magnification) as 0 8 $\pm$ 0 2 μm

In vivo tests of the colloid

The uptake of the Tc S colloid by the liver and bone marrow was tested in rabbits supine under a gamma camera (Nuclear Chicago Pho/Gamma III) Sequential scintigrams were then obtained after intravenous injection of the colloid into the ear vein

A Tc S colloid of good quality is mainly taken up by the liver and the rest by the spleen and bone marrow If however the colloid consists of particles or aggregates larger than 7 μm they will be trapped in the capillary bed of the lung The field viewed by the gamma camera was therefore divided into two parts one part A viewing the cranial part with the chest and the other part B the caudal part including the liver A scintigram was obtained every minute

after administration and the counts were registered separately for fields A and B The ratio (counts in A/counts in B) registered 15 minutes after administration was used as an index of the quality of the colloid This method is similar to that described by DWORKIN et coll who used the liver to blood pool (L/BP) ratio as an index of the quality of the colloid in man A series of photographs with the border between the fields marked with a black line, are given in Fig 4 As the colloid also accumulates in bone marrow, the pelvis and backbones could be seen when the caudal part of the body, except the liver, was viewed by the undivided gamma camera field C As may be seen from Fig 4, the bone marrow accumulation was approximately the same in fields A and C The ratios (counts in C/counts in B, and counts in A/counts in B) would therefore be roughly the same if no particles were trapped by the lungs

The gamma camera has been used for studying the influence of the stabilization agent on the quality of the Tc-S colloid The ratios (counts in A/counts in B) established after 15 minutes are given in Table 1

No significant variation of the quality was found with or without stabilizer This confirms the finding by WEBBER et coll (1969) and LOPEZ & FRENCH (1969) that there was no significant uptake in the lungs We also studied the use of a reduced amount of $Na_2S_2O_3 \cdot 5H_2O$ (1 6 mg) and omitted both the $KReO_4$ and the stabilizer, as suggested by LOPEZ & FRENCH This preparation was administered to a rabbit viewed by the gamma camera The quality value obtained was 0 204, which is significantly higher than for the standard preparation No uptake was observed in the lungs but the increased level of free TcO_4 could possibly explain this figure

The quality of the Tc S colloid also depends on the time interval in boiling water during which the colloid is formed Colloids were therefore prepared at different time intervals, and investigations were carried out in rabbits with a gamma camera as above The counts in A/counts in B, and counts in C/counts in B ratios are given in Table 2 There were no great differences in the quality of these preparations with heating intervals varying from 2 to 10 minutes

The stability of the Tc S colloid was also tested by administering it to rabbits at different times after the preparation and by studying the liver uptake The ratios (counts in A/counts in B) are given in Table 3 The conclusion of this test is that no significant change of the colloid occurred during the day This fact was also confirmed by filtration tests

Conclusion

The aim of this investigation was to make a Tc S colloid for liver and bone marrow scintigraphy free from harmful reactions The preparation procedure

was studied in detail The use of Haemaccel as stabilization agent gives reproducible preparations without any potential risk of pyrogenic or anaphylactic reactions. In vivo tests in rabbits indicate that the Tc S colloid is a good agent for liver and bone marrow scintigraphy and has now replaced the ^{198}Au colloid in our department The chemicals are delivered in sterile kits from the pharmacy and the Tc S colloid is prepared by a hospital physicist Clinical experience so far is limited but the method has worked out satisfactorily in more than 800 patients including several repeated examinations at different time intervals

Acknowledgements

The authors wish to express their deep gratitude to their collaborators too numerous to name individually for their great help in the various stages of the work

SUMMARY

A method of preparing a ^{99}Tc^{m} sulfide colloid for bone marrow and liver scintigraphy is described the aim being to achieve a preparation free from harmful reactions The uptake of the colloid in the bone marrow and liver was investigated in rabbits with a gamma camera The preparation is now in clinical use

ZUSAMMENFASSUNG

Eine Methode zur Herstellung eines Kolloidpraparates von ^{99}Tc^{m} Sulfid fur Knochenmark und Leberszintigraphie wird beschrieben Das Ziel der Arbeit war ein Praparat zu produzieren das frei von schadlichen Nebenwirkungen ist Die Aufspeicherungsrate des Kolloid in Leber und Knochenmark von Kaninchen wurde mit Hilfe einer Gammakamera bestimmt Das Praparat wird jetzt klinisch benutzt

RÉSUMÉ

Les auteurs decrivent une methode de preparation de sulfite de ^{99}Tc^{m} pour la scintigraphie de la moelle osseuse et du foie le but etant de faire une preparation qui ne donne pas de reaction nuisible Ils ont etudie la fixation de ce colloide dans la moelle osseuse et dans le foie sur des lapins au moyen d une gamma camera

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COMPARATIVE STUDY OF DISTRIBUTION OF INJECTED ZINC 65 IN THE MANDIBULAR CONDYLE AND OTHER TISSUES IN RAT AS DETERMINED BY GAMMA SCINTILLATION

by

Bo Bergman

Experiments using radioactive zinc as ^{65}Zn Cl solution have shown that its turnover varies greatly in different tissues The most rapid uptake has been observed in liver pancreas spleen and intestine in rabbits (Molina et coll 1961) and in pancreas liver and spleen in mice rats and dogs (Rubini et coll 1961) In muscles brain and bones the uptake and loss was found to be slow Studies on rats have shown a prolonged retention in bone of intravenously injected radiozinc (Gilbert & Taylor 1956 Ballou & Thompson 1961 Taylor 1961)

High concentrations of zinc have been detected by means of autoradiography in skeletal zones undergoing mineralization, e g in the endochondral growth zones of the long bones (Haumont 1961 Haumont & Vincent 1961 Kinnamon 1963 Haumont 1963) However corresponding data are lacking for the mandibular condyle the most active growth centre of the mandible

The present investigation was undertaken in order to study quantitatively by means of scintillation counting the distribution of ^{65}Zn in the mandibular condyle

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and to compare this distribution with that in some other organs and tissues of female albino rats. The study was performed on both young and adult rats.

Materials and Methods

Experimental animals and their treatment. The experimental animals used in the present study were albino rats of the Sprague Dawley strain. All animals were normally lively and no signs of infection, deficiency or any disease were observed on visual inspection. In order to obviate variability due to prostatic and seminal vesicle uptake (cf. RUBINI et coll. 1961), only females were used. None of the females were pregnant.

The animals were housed in cages of acrylic resin with steel covers and had free access to ordinary tap water and a pellet diet, 210 Anticimex (Anticimex, Norrviken, Stockholm). The animals were kept in the same laboratory room where the temperature varied between 20 and 22° C.

The uptake of radioactive zinc was studied in 3- and 24-week old rats. In both age groups 10 litters were used with 8 rats in each. Each litter was placed in one cage. The survival periods studied were 6, 12 and 24 hours, 2, 4, 5.6, 8 and 11.3 days for the 3-week old animal group and 6, 12 and 24 hours, 2, 4, 8, 16 and 32 days for the 24-week old group. There were 10 rats in each survival period group, making a total of 160 rats. For each of the eight survival periods in the two age groups, one rat was killed from each of the ten litters. By means of this experimental design, the biologic variation among the ten litters was obtained. Another group of 63 rats was used for methodologic studies.

Radioactive zinc. The radioactive zinc, ^{65}Zn, was supplied by the Radiochemical Centre, Amersham, as a $^{65}ZnCl_2$ solution. The specific activities of the two delivered stock solutions were 1.023 and 0.922 mCi/mg Zn, respectively.

The half life of ^{65}Zn is 245 days. Forty-nine per cent of the disintegrations of ^{65}Zn result in the emission of gamma rays with an energy of 1.1156 MeV (LEDERER et coll. 1968). A gamma-sensitive NaI (Tl) scintillation detector was therefore used for the measurements.

Injection solutions were prepared by diluting the original stock solution with physiologic saline. The calculated amount of radiozinc given per gram body weight was 0.2 μCi.

The amount of inactive (non-radioactive) zinc given varied between 0.20 and 0.22 μg per gram rat due to the different specific activities of the two delivered stock solutions. According to Handbook of Toxicology 1956 (SPECTOR p. 316), the lethal dose in rats for intravenously administered zinc chloride is 60 to 90 μg/g rat. Obviously, the dose of inactive zinc given in the present study was small.

Table 1

Comparison between intraperitoneal injection (i p) and subcutaneous injection (s c) of ^{65}Zn after 6 and 24 hours — The mean concentration ($\bar{x}$) from 6 rats and standard deviation (s) in per cent ($s_0 = s \times 100/\bar{x}$) are indicated — 3-week-old female rats were used

Tissue	6 hours				24 hours			
	i p		s c		i p		s c	
	$\bar{x}$	s_0	$\bar{x}$	s_0	$\bar{x}$	s_0	$\bar{x}$	s_0
Blood	0 18	7	0 19	12	0 19	10	0 18	10
Kidney	3 03	15	3 11	12	1 73	2	1 67	17
Pancreas	3 99*	7	3 18	20	1 83	25	2 04	47
Spleen	2 05	7	1 90	7	1 68	10	1 51	12
Liver	5 18	15	5 80	27	2 25	10	2 13	25
Heart	0 82	10	0 80	7	1 03	10	0 95	20
Incisors	0 40	44	0 39	47	0 57	7	0 52	27
Mandibular condyl	3 66	29	3 68	25	5 45	10	4 70	27
Mandibular bone	2 02	12	1 98	22	2 68	17	2 50	25
Tibia epiphysis	1 30	22	1 51	17	2 16	24	1 92	15
Tibia diaphysis	1 14	7	1 16	17	1 56	12	1 67	24

* Difference 3 99—3 18 almost significant ($p < 0\ 05$)

Administration of the radioactive solution Two routes of administration of the radioactive zinc intraperitoneal and subcutaneous injections were found to be suitable Subcutaneous injections were given in the neck region A comparison of the results with subcutaneous and intraperitoneal injections is shown in Table 1 Differences were tested by means of Student s t test

With one exception no significant differences were found between the two methods of administration of the radioactive solution at 6 and 24 hours post injection At 6 hours an almost significant difference ($p < 0\ 05$) was found for pancreas However it seems probable that by 6 hours post injection no accumulation of activity in the abdominal organs could be ascribed to direct contamination from the injected intraperitoneal deposit Intraperitoneal injections were chosen in the present investigation

A 1 ml syringe calibrated in units of 0 01 ml was used in all injections Hypodermic needles of size No 20 were used The radioactive solution was injected slowly Leakage or backflow of the injected solution occurred only rarely and when either occurred the animal was discarded from the experimental series (cf Table 4)

The influence of the following three factors on the uptake of radioactive zinc

Table 2

Concentrations of ^{65}Zn in relation to the amount of ^{65}Zn, the amount of Zn carrier and the volume intraperitoneally injected 6 hours after administration — The mean concentration ($\bar{x}$) from 6 rats and standard deviation (s) in per cent ($s\% = s \times 100/\bar{x}$) are indicated — 3-week-old female rats were used

	Administered per gram body weight							
^{65}Zn μCi	0.1 μCi		0.2 μCi		0.2 μCi		0.2 μCi	
Zn carrier μg	0.1 μg		0.2 μg		0.2 μg		2.2 μg	
Volume ml	0.01 ml		0.01 ml		0.02 ml		0.01 ml	
Tissue	$\bar{x}$	s %	$\bar{x}$	s %	$\bar{x}$	s %	$\bar{x}$	s %
Blood	0.17	15 %	0.14	27 %	0.16	10 %	0.15	17 %
Kidney	2.45[1]	15 %	1.78[2]	22 %	2.28	12 %	1.97	20 %
Pancreas	5.58	27 %	3.59	37 %	3.72	37 %	5.86	54
Spleen	1.72[3]	17 %	1.57	47 %	1.52[4]	7	1.16[5]	15
Liver	4.31[6]	10 %	3.06[7]	25 %	4.76[8]	22 %	3.25[9]	20
Heart	0.64	25	0.53	22 %	0.67	32 %	0.50	15
Incisors	0.38	64 %	0.26	42 %	0.30	44 %	0.36	47 %
Mandibular condyle	2.93	15 %	2.55	39 %	2.52	12 %	2.53	27 %
Mandibular bone	1.29	22	1.18	27	1.27	22 %	1.21	12 %
Tibia epiphysis	1.22	12 %	1.09	32 %	1.19	15 %	1.48	47 %
Tibia diaphysis	0.96	15	0.79	27 %	0.91	30 %	0.85	20 %
Hair	0.17	91 %	0.16	64	0.15	37 %	0.25	49 %

Almost significant ($p \leq 0.05$) differences were found between 1—2, 6—7, 7—8, 8—9. Significant ($p < 0.01$) differences were found between 3—5, 4—5.

was also studied: (1) the amount of radioactive zinc, (2) the amount of inactive zinc carrier and (3) the volume of the injected solution.

Three week old females were killed 6 hours after the injection, corresponding to the shortest survival period used in the present study. The contents of the injection solutions used for each group and the resulting tissue activity concentrations are presented in Table 2. Differences were tested by means of Student's t test.

Some almost significant differences ($p \leq 0.05$) were observed between the groups for liver and kidney, and the ^{65}Zn concentration in the spleen in the last column of Table 2 differed significantly ($p < 0.01$) from the corresponding values in the first and third columns. However, the distribution patterns were similar in the four groups and no significant differences were found for blood, pancreas, heart, incisors, or bone tissues. As already mentioned, the calculated amount of radiozinc given per gram rat in the present study was 0.2 μCi. The amount of inactive zinc varied between 0.20 and 0.22 μg per gram rat.

Table 3

Concentrations of ^{65}Zn 24 hours after intraperitoneal injection in relation to two slightly different sizes of some specimens — The mean ($\bar{x}$) from 6 rats and standard deviation (s) in per cent ($s\,\% = s \times 100/\bar{x}$) are indicated — 8-week-old female rats were used

Smaller specimen	$\bar{x}$	s %	Larger specimen	$\bar{x}$	s %
Right incisors	1 23	20	Left incisors	1 0[illegible]	4[illegible]
Right mandibular condyle	18 70	29	Left mandibular condyle	20 19	25
Right mandibular bone	6 63	42	Left mandibular bone	7 06	44
Right tibia diaphysis	4 07	34	Left tibia diaphysis	3 91	22
Right tibia epiphysis	9 98	2[illegible]	Left tibia epiphysis	9 21	17

Sampling techniques At the end of the various survival periods, the rats were slightly anaesthetized with ether and sacrificed by decapitation The following pattern of sampling was followed throughout the study Approximately 0 8 ml blood was collected from each rat immediately after decapitation The various tissues to be studied were then rapidly excised in the following order kidney pancreas spleen liver heart teeth mandibular condyle mandibular bone tibia epiphysis tibia diaphysis and hair The tissues were not perfused When possible the whole organ was taken for measurement The liver sample removed was the median lobe The teeth examined were the four incisors which were broken off at the gingival margin All visible pulp was removed and unseparated enamel and dentin were analysed The mandibular bone samples consisted of the ramus and part of the corpus including both spongy and compact bone the periosteum was scraped away In removing the tibia diaphysis great care was taken to avoid the metaphyseal part of the long bone Attempts were made to collect only the compact bone of the diaphysis Therefore the diaphyseal part was dissected free sectioned along its long axis and freed of visible bone marrow and periosteum The hair analysed consisted of the shafts cut off as near the epidermis as possible The hair roots were not analysed The organ or tissue to be investigated was placed in a stoppered glass tube immediately after removal The wet weight of the sample was recorded

A careful and uniform dissection technique will minimize the errors However when only a portion of a tissue is removed some errors may result from the dissection technique These should be reduced when whole organs or well defined portions of tissues are taken In the dissection of some tissues it is difficult to observe well defined anatomical landmarks An experiment was carried out to demonstrate the error due to sample size variation The assumption was made that the distribution of ^{65}Zn was equal in comparable specimens from

Table 4

Concentration of ^{65}Zn in the mandibular condyle and in selected hard and soft tissues of 3 week old female rats various time periods after intraperitoneal injection — Mean ($\bar{x}$) from 10 rats and standard deviation (s) are indicated s% = s × 100/$\bar{x}$

Tissue	6 hours			12 hours*			24 hours		
	$\bar{x}$	s	s %	$\bar{x}$	s	s %	$\bar{x}$	s	s %
Blood	0 25	0 03	12 %	0 19	0 04	21 %	0 20	0 02	10 %
Kidney	3 06	0 36	12 %	2 35	0 32	14 %	1 94	0 23	12 %
Pancreas	6 06	1 06	17 %	4 39	1 93	44 %	2 70	0 71	26 %
Spleen	2 45	0 38	16 %	2 02	0 23	11 %	1 84	0 22	12 %
Liver	5 12	0 86	17 %	3 03	0 78	26 %	2 35	0 51	22 %
Heart	0 83	0 11	13 %	1 02	0 12	12 %	1 04	0 14	13 %
Incisors	0 28	0 07	25 %	0 37	0 11	30 %	0 44	0 08	18 %
Mandibular condyle	2 61	0 40	15 %	3 13	0 57	18 %	4 12	0 65	16 %
Mandibular bone	1 43	0 15	10 %	2 00	0 46	23	2 37	0 34	14 %
Tibia epiphysis	1 39	0 44	32 %	1 47	0 25	17 %	1 82	0 41	23 %
Tibia diaphysis	0 99	0 08	8 %	1 28	0 24	19 %	1 46	0 17	12 %
Hair	0 04	0 04	100 %	0 06	0 03	50	0 05	0 05	100 %

the right and left side of any single rat Two slightly different sized samples of incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis from each of six rats were used The uptake of radiozinc was compared between the two specimens The results are given in Table 3 Intra individual differences were tested by means of Student s t test

No significant differences were observed in the uptake of ^{65}Zn between the larger and smaller specimens Hence, a small inaccuracy in the dissection technique used for these samples probably caused no significant error in the calculation of the uptake

The weighing procedure itself may cause an error in the calculation of the ^{65}Zn concentration The mean weight of the tissues to be measured varied between approximately 15 mg (mandibular condyle and incisors) and 600—800 mg (blood and liver) The maximum difference obtained during the entire investigation period between two weighings of the same test tube was 0 3 mg For an organ weighing 15 mg this means a maximal error of 2 %, which does not appear to influence the calculated ^{65}Zn concentration value for a small tissue to any noticeable degree

A further experiment was carried out to study whether the weighing error would cause a considerable error in the calculation of the uptake of ^{65}Zn in

Table 4 *(cont.)*

2 days			4 days			5 6 days			8 days			11 3 days		
$\bar{x}$	s	s	$\bar{x}$	s	s°	$\bar{x}$	s	s°	$\bar{x}$	s	s	$\bar{x}$	s	s
0 16	0 03	19 *	0 17	0 02	12	0 13	0 01	8	0 08	0 01	13	0 07	0 02	29 *
0 97	0 20	21	1 04	0 06	6	0 56	0 07	13	0 30	0 02	7	0 23	0 06	26 *
1.20	0 20	17 *	1 23	0 90	73	0 62	0 04	6 *	0 35	0 03	9 *	0 32	0 06	19
1 00	0 19	19	0 87	0 10	11	0 60	0 04	7	0 28	0 02	7 *	0 21	0 05	24 *
1 24	0 29	23 *	1 25	0 15	12	0 72	0 07	10 *	0 48	0 06	13	0 38	0 12	32 *
0 71	0 17	24	0 67	0 04	6	0 51	0 03	6	0 26	0 02	8	0 20	0 05	25
0 49	0 15	31	0 92	0 17	18	1 00	0 14	14	1 15	0 12	10	1 43	0 20	14
3 48	0 66	19 *	4 03	0 45	11	3 28	0 51	16	1 55	0 29	19	1 21	0 46	38
2 66	0 62	23	3 32	0 33	10	3 31	0 48	15 *	2 57	0 31	12	2 53	0 36	14
1 46	0 38	26	2 10	0 40	19	1 97	0 32	16 *	1 16	0 14	12	1 18	0 20	17
1 73	0 49	28	2 24	0 13	6	2 16	0 20	9	2 15	0 38	18	2 32	0 37	16
0 04	0 02	0	0 11	0 03	27	0 06	0 03	50 *	0 06	0 04	67	0 08	0 08	100 *

* Mean from nine rats one rat discarded due to backflow of the injected radioactive solution

small tissues as compared to large tissues Three rats were administered radio zinc intraperitoneally *Twenty four hours after* injection the rats were killed by decapitation Blood was collected from each rat and divided in five to six portions varying in amounts from 15 mg up to 800 mg The samples were measured under *standard conditions* Comparisons did not show any significant differences between the ^{65}Zn uptake per milligram blood in the *various amounts of blood* Blood was chosen for this experiment because it is the only tissue where it can be assumed a priori that ^{65}Zn is homogenously distributed From this experiment it seems logical to assume that the small *size of* e g the mandibular condyle and incisors will not cause any significant error in the calculation of the uptake of ^{65}Zn for respective organs with the techniques used Thus the calculated ^{65}Zn concentrations of mandibular *condyles and* incisors appear to be *comparable* to those calculated for large samples such as blood liver and kidney

In preliminary experiments fat tissues showed a very low uptake of ^{65}Zn The concentration of ^{65}Zn in a tissue e g heart, spleen or kidney might be influenced by *the presence of fat tissue* In the dissection great care was taken to avoid fat tissue However as this dissection error cannot be completely eliminated or its size estimated it is included in the total variation

Table 5

Concentration of ^{65}Zn in the mandibular condyle and in selected hard and soft tissues of 24 week old female rats various time periods after an intraperitoneal injection — Mean ($\bar{x}$) from 10 rats and standard deviation (s) are indicated $s\% = s \times 100/\bar{x}$

Tissue	6 hours			12 hours			24 hours		
	$\bar{x}$	s	s %	$\bar{x}$	s	s %	$\bar{x}$	s	s
Blood	2.05	0.17	8%	2.18	0.15	7%	2.17	0.19	9%
Kidney	28.95	2.37	8%	24.80	2.12	9%	22.86	2.75	12%
Pancreas	32.57	5.79	18%	22.07	5.45	25%	15.46	4.56	29%
Spleen	21.11	1.80	9%	20.38	1.20	6%	19.94	1.49	7%
Liver	27.58	2.75	10%	25.61	2.76	11%	25.15	4.17	17%
Heart	7.33	0.73	10%	8.61	0.39	5%	10.60	0.84	8%
Incisors	0.45	0.09	20%	1.04	0.47	45%	1.24	0.39	31%
Mandibular condyle	5.69	1.01	18%	7.99	0.87	11%	8.73	0.95	11
Mandibular bone	2.02	0.35	17%	2.55	0.36	14%	3.18	0.45	14
Tibia epiphysis	5.31	0.60	11%	5.72	0.43	8%	6.62	1.26	19%
Tibia diaphysis	2.04	0.54	26%	2.92	0.40	14%	3.72	0.78	21%
Hair	0.25	0.25	100%	0.64	1.17	183%	1.01	1.08	107%

Techniques in scintillation measurements The techniques used for scintillation measurements were uniform and performed according to the following scheme Immediately after weighing of the samples in their glass tubes, concentrated hydrochloric acid was added up to a certain level of the tube After being immersed 48 hours in the hydrochloric acid solution the samples were completely disintegrated and, hence, all samples counted possessed an almost identical geometry at a volume of approximately 3 ml The glass tubes, sealed with rubber stoppers and containing the organs to be investigated, fitted to the well of the scintillation crystal The dimensions of the glass tubes used were nearly identical

The sample geometry may influence the counting rate in the scintillation detector A simple experiment was performed to demonstrate possible changes in counting rate and, thus, ^{65}Zn concentration due to variation in the sample geometry caused by variation in the amounts of hydrochloric acid added to the samples It was found that the number of impulses recorded was not significantly influenced by a change in the total volume between 2 7 and 3 3 ml obtained by adding various amounts of hydrochloric acid This volume range represents the extreme values for volumes used in the present study

The gamma ray measurements were performed in a Tracerlab scintillation detector P-20DW with a well type crystal The size of this NaI (Tl) crystal was 3″ × 3″ with a well of 1″ × 2″ The photomultiplier tube was connected to a

Table 5 *(cont)*

2 days			4 days			8 days			16 days			32 days		
$\bar{x}$	s	s	$\bar{x}$	s	s	$\bar{x}$	s	s	$\bar{x}$	s	s	$\bar{x}$	s	s
2 33	0 13	6	1 64	0 04	2	1 27	0 19	15 °	0 90	0 05	6	0 20	0 04	20 °
15 99	0 77	5	8 27	0 55	7	5 13	0 43	8	2 73	0 23	8	0 57	0 11	19
9 23	3 79	41	5 83	2 13	37	4 88	1 02	21	2 15	0 75	35	0 63	0 20	32
15 14	0 82	5	7 84	0 44	6	4 57	0 32	7	2 44	0 18	7	0 52	0 11	21
19 00	1 39	7	10 56	1 03	10	7 28	0 58	8	3 40	0 27	8	0 62	0 10	16
9 51	0 56	6	5 72	0 34	6	3 76	0 ?1	6	2 16	0 23	11	0 51	0 07	14
1 56	0 42	27	1 68	0 31	18	3 64	0 40	11	7 22	1 52	21	9 24	1 91	21 °
10 66	0 87	8	11 66	2 17	19	14 17	2 34	17	12 3)	2 85	23	6 40	1 26	20
3 84	0 51	13	3 77	0 44	12	4 51	0 46	10	4 94	0 68	14	3 45	0 57	17
6 /0	1 16	17	4 73	0 49	10	5 00	1 ?3	25	4 47	1 28	29	1 88	0 90	48
4 42	0 93	21	3 14	1 34	43	4 01	0 99	25	3 50	1 25	36 °	1 30	0 58	43 °
2 22	1 8?	82	0 40	0 33	83	1 79	2 01	112	6 18	8 82	143	2 58	2 76	107 °

Tracerlab Sc 73 Versa/Matic II Scaler The operating voltage was 1 200 V which was proved to be the lowest voltage giving satisfactory sensitivity The equipment was placed in a room with constant temperature and humidity and was never turned off

Between 4 000 and 10 000 impulses were recorded for each sample The back ground was determined each experimental day The counts min [1] g [1] sample was calculated after subtraction of the background counting rate The uptake of [6] Zn in a tissue was expressed as the concentration which is defined as the counts min [1] g [1] tissue divided by the counts min [1] injected per gram body weight A comparison was made between specimens and standard solutions

Results

The results are presented in graphs and tables Intra individual differences were tested by means of Student s t test In general it was found that the stand ard deviation in per cent of the mean was large for the hair samples it was extremely large (Tables 4 and 5)

3 week old rats The results for these are given in Fig 1 and Table 4 After 6 hours the concentration of [6] Zn in blood decreased very slowly It was found

Table 5

Concentration of ⁶⁵Zn in the mandibular condyle and in selected hard and soft tissues of 24 week-old female rats various time periods after an intraperitoneal injection — Mean ($\bar{x}$) from 10 rats and standard deviation (s) are indicated $s\,\% = s \times 100/\bar{x}$

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The gamma ray measurements were performed in a Tracerlab scintillation detector P 20DW with a well type crystal The size of this NaI (Tl) crystal was 3″ × 3″ with a well of 1″ × 2″ The photomultiplier tube was connected to a

that the pancreas liver and kidney in the earliest survival periods studied showed the highest concentrations significantly higher ($p < 0.001$) than all the hard tissues at 6 hours In these organs, as in the other two soft tissues the spleen and heart the concentration of radiozinc fell comparatively rapidly After two days the hard tissues dominated the distribution pattern The incisors were the only one of the tissues studied showing an increasing ^{65}Zn concentration during the entire experimental period Of the skeletal samples the mandibular condyle initially showed the highest ^{65}Zn uptake after 5 6 days however, it was surpassed by mandibular bone and tibia diaphysis After 5 6 days all the hard tissues had significantly higher ^{65}Zn concentrations than the soft tissues ($p < 0.001$)

24 week old rats The results for these are given in Fig 2 and Table 5 The concentration of ^{65}Zn in blood was about the same from 6 hours up to 2 days and thereafter decreased slowly At early survival times the soft tissues showed the highest uptake of all the tissues studied The kidney pancreas, liver spleen and heart differed significantly ($p < 0.001$) from all hard tissues at 6 12 and 24 hours with some exceptions The soft tissue concentrations fell steadily and at 16 and 32 days post injection the hard tissues and the hair dominated the distribution pattern At 32 days all the hard tissues differed significantly from all the soft tissues As in the 3 week animals the mandibular condyle initially showed the highest ^{65}Zn concentration of the skeletal tissues and at 32 days it still showed a significantly higher uptake than any of the other tissues except the incisors The incisors were the only tissue which steadily increased the radiozinc concentration during the experimental period

Discussion

The concentration values of ^{65}Zn obtained for the various tissues include experimental errors as well as biologic variations Several of the experimental errors which might influence the results are analysed and discussed under the heading materials and methods These experimental errors were relatively small compared to the total variation obtained under similar conditions Therefore the total variation expressed as standard deviation should be approximately representative of the biologic variation

Comparing the results obtained for the young rats with those of the adults it is found that the turnover (uptake and loss) of radiozinc was faster in the young rats However the pattern of distribution was, in general similar for

Legend to Fig 1 (see opposite page)
Variation with time in the concentration of ^{65}Zn in 3 week old female rats after an intraperitoneal injection Each value indicates the mean from ten rats (at 12 hours 2 days and 5 6 days means from nine rats) The standard deviations are presented in Table 4

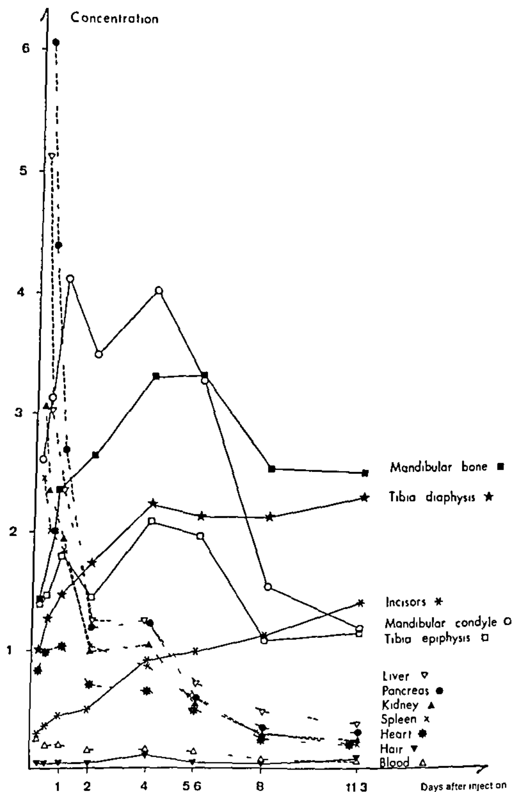


Fig 1 (for legend see opposite page)

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Discussion

The concentration values of ^{65}Zn obtained for the various tissues include experimental errors as well as biologic variations Several of the experimental errors which might influence the results are analysed and discussed under the heading materials and methods These experimental errors were relatively small compared to the total variation obtained under similar conditions Therefore the total variation expressed as standard deviation should be approximately representative of the biologic variation

Comparing the results obtained for the young rats with those of the adults it is found that the turnover (uptake and loss) of radiozinc was faster in the young rats However the pattern of distribution was in general similar for

Legend to Fig 1 (see opposite page)

Variation with time in the concentration of ^{65}Zn in 3 week-old female rats after an intraperitoneal injection Each value indicates the mean from ten rats (at 12 hours 2 days and 56 days mean from nine rats) The standard deviations are presented in Table 4

the two age groups. These observations are in accordance with BALLOU & THOMPSON (1961) and STRAIN et coll. (1964a and 1964b). The highest turnover of radiozinc was observed in pancreas, liver, kidney, and spleen. In hard tissues and hair, ^{65}Zn was deposited more slowly and bound for a relatively long time (Figs. 1 and 2, Tables 4 and 5). This general picture of ^{65}Zn distribution is in good agreement with previous scintillation studies (SHELINE et coll. 1943a, FEASTER et coll. 1955, WAKELEY et coll. 1960, RUBINI et coll. 1961, BALLOU & THOMPSON 1961, MOLINA et coll. 1961, CZERNIAK et coll. 1962, STAND et coll. 1962, ROBERTSON & BURNS 1963, RIBAS et coll. 1963, STRAIN et coll. 1964a and 1964b, SPENCER et coll. 1965, JOHNSTON et coll. 1966). The results obtained in the present study are also in good agreement with those of a preceding study on mice by means of whole body autoradiography (BERGMAN & SOREMARK 1968).

In the present experiment, the early accumulation of ^{65}Zn in the tissues was not followed. In the first survival period studied, 6 hours post injection, the whole blood ^{65}Zn concentration was low compared to both soft and hard tissues. After six hours, the radiozinc concentration in blood fell rather slowly. It has been reported that intravenously injected ^{65}Zn rapidly disappeared from blood and after a few hours the major part of radiozinc had left the blood (SHELINE et coll. 1943a, DENNES et coll. 1962, STAND et coll. 1962, BRAHMANANDAM et coll. 1965, JOHNSTON et coll. 1966).

A high initial uptake of ^{65}Zn in pancreas has been noted previously (e.g. SHELINE et coll. 1943a, WAKELEY et coll. 1960, MOLINA et coll. 1961, CZERNIAK et coll. 1962). Some of the ^{65}Zn uptake may be related to enzymatic (pancreatic carboxypeptidase) and hormonal (insulin) processes within the organ. The physiologic relationship between zinc and insulin has been the subject of several investigations (cf. VALLEE 1959). MILLAR et coll. (1961) studied the autoradiographic localization of ^{65}Zn 24 hours post injection in rats fed a zinc deficient diet. In the pancreas, the highest concentration was found in the islets of Langerhans. It has been assumed that zinc is concerned with the occurrence *of storage and secretion of insulin in the* β *cells* (cf. VALLEE 1959). BOQUIST & LERNMARK (1969) reported a decreased granulation of the islet β cells of zinc deficient hamsters. However, it does not seem likely that all the ^{65}Zn uptake in pancreas can be associated with pancreatic carboxypeptidase and insulin. Zinc is normally excreted mainly by the feces, primarily by way of the pancreatic secretions. The high initial uptake of radiozinc in the pancreas has been related mainly to this fact (MONTGOMERY et coll. 1943, BIRNSTINGL et coll. 1956, ROBERTSON & BURNS 1963).

Several studies have shown that the urinary excretion of ionic zinc is low (SHELINE et coll. 1943b, MOLINA et coll. 1961, ROBERTSON & BURNS 1963, SPENCER et coll. 1965, BRAHMANANDAM et coll. 1965, ANDRASI & FEHER 1967).

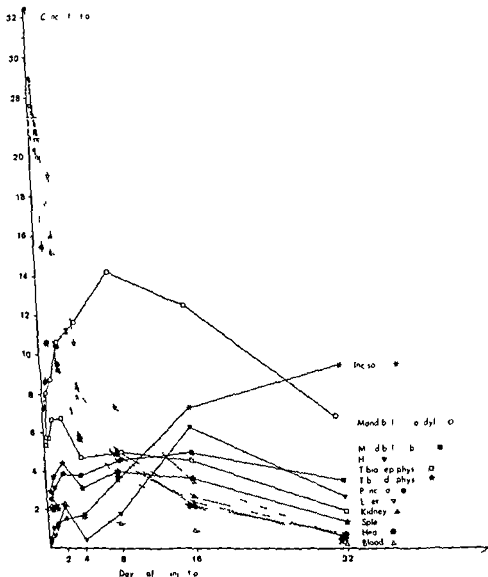


Fig Variation with time in the concentration of [6] Zn in 4 week-old female rats after intra peritoneal injection Each value indicates the mean from ten rats The standard deviat ons are gi en n Table 5

The rapid accumulation of [6] Zn in the kidney has therefore been considered to be due to the rich vascularization of this organ to the presence of carbonic

anhydrase, and to association of zinc with proteins (STAND et coll 1962, SPENCER et coll 1965)

The high initial uptake of ^{65}Zn in the liver and the spleen may be due to the fact that these organs are very rich in blood STAND et coll (1962) proposed that the high ^{65}Zn uptake in liver should also be related to incorporation of ^{65}Zn into some of the liver enzymes, e g alcoholic dehydrogenase

The concentration in the soft tissues decreased after six hours except for the heart, where, in both groups, the concentration fell after 24 hours SPENCER et coll (1965) found a higher uptake and retention of radiozinc in cardiac muscle compared to skeletal and diaphragmatic muscles and suggested this was due to a higher content of the zinc metalloenzyme lactic dehydrogenase in the cardiac muscle as compared to other muscles The role of zinc in muscle contraction has been discussed by ISAACSON & SANDOW (1963)

An uptake of radiozinc in hair shafts was noted especially in the 24 week old animals It should be noted that the standard deviation in per cent of the mean was extremely large for hair That hair accumulates zinc over long periods has been reported by e g GILBERT & TAYLOR (1956), BALLOU & THOMPSON (1961) and STRAIN et coll (1964a and 1964b) The accumulation of radiozinc in the hair shafts indicates the growth of the hair Once incorporated, the zinc in hair shafts does not seem to be available for exchange (MACAPINLAC et coll 1966) In this connection it is interesting to note that a zinc containing protein is active in the biosynthesis of tyrosine melanin at the site of dopachrome of the pigment production in melanocytes (FITZPATRICK 1965) CARLSSON & SOREMARK (personal communication) have found very high concentrations of zinc and copper in hyperpigmented tissues FITZPATRICK et coll (1965) have discussed the unusually high concentration of melanocytes in hair follicles The uptake of zinc in hair could possibly reflect the protein binding of zinc in melanocytes and the high zinc concentration in melanosomes (premelanosomes for albinos)

Studies indicate that the zinc concentration in bone is directly related to the degree of mineralization (ALEXANDER & NUSBAUM 1962, BERGMAN 1970a) BRUDEVOLD et coll (1963) found that zinc was readily acquired by synthetic hydroxyapatite, competing with calcium for position on the surface of the apatite crystals Mineralized tissues have shown a great capacity of accumulating zinc (HUXLEY & LEAVER 1966) SAMACHSON et coll (1967) reported that bone mineral showed a greater and more rapid uptake of ^{65}Zn than the bone matrix

Of the hard tissues, only the incisors steadily increased in ^{65}Zn concentration in both age groups over the experimental periods The crowns of the rat incisors are almost fully mineralized at 3 weeks of age and are subject to continuous growth and abrasion Incorporated into the body of the crystals of the rat incisors during mineralization, ^{65}Zn is probably lost mainly due to abrasion of the incisal

edges Normally no remodelling takes place in the enamel or dentine As the growth of the incisors proceeded, the 65 Zn concentration in the crowns increased Studies by BAUER et coll (1961) and BAUER & SHTACHER (1968) using 45 Ca and 85 Sr indicated that the mineral in rat incisors is less accessible to exchange than the bone mineral

Earlier studies have shown that 65Zn is taken up by the skeleton at a low rate and is bound for long periods (SHELINE et coll 1943a, GILBERT & TAYLOR 1956 WAKELEY et coll 1960, TAYLOR 1961 BALLOU & THOMPSON 1961, STAND et coll 1962 RICHMOND et coll 1962 CZERNIAK et coll 1962 STRAIN et coll 1964a and 1964b) The bone examined in previous studies has mostly been femur or tibia In those cases where direct comparisons are possible (SHELINE et coll 1943a WAKELEY et coll 1960, BALLOU & THOMPSON 1961, RICHMOND et coll 1962 CZERNIAK et coll 1962, STRAIN et coll 1964 and 1964b), the general turnover picture of 65 Zn obtained in the present study for mandibular bone and tibia samples appears to be in good agreement with previous studies Some minor differences between the studies may be explained by variations in the methods used

The 65 Zn uptake in the mandibular bone of the 3 week old animals was initially somewhat higher than in the tibia diaphysis From 8 days on the difference was considerably reduced but was almost significantly higher even at 11 3 days In the tibia epiphysis the initial uptake was about the same as in the tibia diaphysis After 5 6 days a loss took place through the eighth day There after until 11 3 days post injection the curves of the mandibular bone the tibia diaphysis and epiphysis were running approximately parallel with one another In the 24 week old animals the uptake of 65 Zn in the tibia epiphysis was higher than in the mandibular bone and the tibia diaphysis up to 4 days post injection At 8 and 16 days no significant differences were found between the three skeletal samples but at 32 days the mandibular bone showed a significantly higher ($p < 0.001$) value than the tibia samples The curves for tibia samples (Figs 1 and 2) agree rather well with a curve on 85Sr uptake in rat tibia presented by BAUER & SHTACHER (1968)

Of the skeletal tissues the mandibular condyle in both age groups initially showed the most rapid uptake of 65 Zn From the first to the fourth day after injection the condyle in the young group showed the highest concentration of all the tissues studied (Fig 1) and in the older group it had the highest concentration from 4 to 16 days (Fig 2) Microautoradiographic studies on the mandibular condyle of young rapidly growing rats (BERGMAN 1970b) showed that 65 Zn was found primarily in the calcifying cartilage and in numerous trabeculae of the spongiosa while in the rest of the cartilage only a low concentration could be detected

At the age of 3 weeks, the rat mandibular condyle is still composed of a comparatively large portion of cartilage. With increasing age, the cartilage in the condyle is gradually replaced as bone is formed. In adult rats this soft tissue constitutes only a thin layer on the top of the condyle (COLLINS et coll. 1946, CUNAT et coll. 1956). CUNAT et coll. reported that between 15 and 19 days after birth remodelling resorption begins on the medial and lateral walls of the rat condyle and that this resorption is quite marked at the age of 23 days. The rapid turnover of ^{65}Zn in the mandibular condyle of the 3 week old animals is probably due to the rapid growth and mineralization which takes place in the condyle at this age. The total zinc concentration in the mandibular condyle of 3 week old rats has been calculated to be about the same as in the incisors, mandibular bone and tibia diaphysis (BERGMAN 1970a). That the turnover of ^{65}Zn was more rapid in the mandibular condyle than in the other skeletal tissues of 3 week old rats indicates that ion exchange and growth take place at a higher rate in the mandibular condyle than in the mandibular bone and the tibia diaphysis at this age.

In the 24 week old animals, the initial uptake of ^{65}Zn was much more marked in the mandibular condyle than in the other hard tissues. The subsequent loss of ^{65}Zn took place somewhat more rapidly in the condyle compared to the other bone samples. However, the turnover was slower than in the 3 weeks animals. The total zinc concentration in the mandibular condyle of 24 week old rats was calculated to be of about the same range as for mandibular bone, tibia diaphysis, and tibia epiphysis (BERGMAN 1970a). The growth and remodelling processes in the condyle are slow in adult rats (CUNAT et coll. 1956). Therefore, the present study seems to indicate that the comparatively rapid turnover of ^{65}Zn in the mandibular condyle of adult rats largely can be ascribed to ion exchange of zinc. This exchange seems to take place at a higher rate in the condyle than in the other skeletal tissues studied. The condyle contains a large number of trabeculae which constitute a large total crystal surface for ion exchange.

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SUMMARY

The distribution of ^{65}Zn in young and adult rats was studied by scintillation measurements. The most rapid turnover was observed in pancreas, liver, kidney and spleen. In hard tissues and hair ^{65}Zn was deposited more slowly. The incisors steadily increased their ^{65}Zn concentration over the experimental period in both age groups. Of the skeletal tissues the mandibular condyle showed a more rapid turnover than mandibular bone, tibia diaphysis or tibia epiphysis.

ZUSAMMENFASSUNG

Die Verteilung von ^{65}Zn in jungen und erwachsenen Ratten wurde durch Scintillationsmessung bestimmt Der rascheste Umsatz wurde in Pankreas Leber Niere und Milz gefunden In den harten Geweben und den Haaren wurde ^{65}Zn langsamer abgelagert Die Inzisoren beider Altersgruppen steigerten ihre ^{65}Zn Konzentration wahrend der Untersuchungszeit standig Von den Skelettgeweben zeigten die mandibularen Condylen einem rascheren Umsatz als die mandibulare Knochensubstanz die Diaphyse oder Epiphyse der Tibia

RÉSUMÉ

L auteur a étudie par mesure de scintillation la distribution du ^{65}Zn chez les rats jeunes et adultes Le turnover le plus rapide a éte observe dans le pancreas le foie le rein et la rate Dans les tissus durs et les poils le ^{65}Zn s est fixé plus lentement Les incisives ont augmente regulierement leur teneur en ^{65}Zn pendant la période d experience dans les deux groupes d age Parmi les tissus squelettiques le condyle mandibulaire presente un turnover plus rapide que l os mandibulaire la diaphyse ou l epiphyse du tibia

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FROM THE DIVISION OF RADIOBIOLOGY, RESEARCH INSTITUTE OF NATIONAL DEFENCE, SUNDBYBERG, SWEDEN

GENETIC EFFECTS OF ^{90}Sr ON VARIOUS STAGES OF SPERMATOGENESIS IN MICE

by

H. FROLÉN

LÜNING et coll. (1963) in two short communications reported that ^{90}Sr injected into male mice increased the rate of intrauterine death in their offspring, both in matings the first 5 weeks after the injection as well as in the 11th to 15th weeks, i.e. cells in post meiotic or meiotic stages and spermatogonial stages, respectively. Furthermore, there were indications that dominant deleterious effects also appeared in following generations. These latter results were rather puzzling and needed further investigation.

The idea that ^{90}Sr might possibly produce genetic effects was based upon the suggestion of MAZIA (1954) that bivalent ions were constituents of the chromosomes. We therefore also examined the effects of ^{45}Ca, besides repeating tests with ^{90}Sr. The preliminary impression from these latter experiments led to the inclusion of tests in which inactive Ca ions were added to the ^{90}Sr. The question as to whether the intraperitoneal injections had produced local effects was investigated by comparing the effects of parallel injections given intravenously. This paper will deal with the results of these tests.

Material and Methods. Males, aged 60 to 70 days, of an inbred CBA strain of mice were given 0.2 ml injections of the solutions listed in Table 1. The mice

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Table 1

Experimental schedule relating to the activity and concentrations of the solutions injected into male mice in the different test series

Roman numbers refer to text sections dealing with resp experimental series	Isotope with or without inactive salt	Amount distributed per animal	
		μCi	mmoles
I II	Sr	20	0 46 10^{-}
III	*Sr+*	*20*	*0 46 10^{-}*
	inactive Ca	—	+0 04
IV	Ca	*16 7*	0 107 toxic
IV	Ca	10	0 064
IV	^{4}Ca	20	0 45 10^{-}
IV	Ca +	20	0 45 10^{-}
	inactive Ca	—	+0 04

were grouped in two series as we were interested in the intrauterine death rate in offspring sired at different times after intravenous and respectively intra peritoneal injection the males were mated *individually to three females per* week The routine mating scheme was

1st to 5th week matings of 3 females/male/week
6th to 9th week no matings
10th to 15th week matings of 3 females/male/week

Certain deviations from this general scheme in individual test series will be mentioned in the presentation of the results

The females were 65 to 75 days old at mating and all except those from the 10th week were killed on the 17th day after the start of mating The uterine content was analyzed and recorded The females in matings the 10th week *after* injection were allowed to give birth to their litters in order to test the F_1 or F_2 generations

It may be pointed out in this connection that all estimates of the possible *effects* of ^{90}Sr or ^{4}Ca are based on comparisons with a concomitant control series in which the males were given saline injections (*sterile 0 9 %* NaCl)

Results

I Effects of intraperitoneal injection of ^{90}*Sr* The main task has been to confirm the earlier results (LUNING et coll 1963) of the mutagenic effects of ^{90}Sr The effects in various stages of spermatogenesis were of *special interest* The routine technique described produced samples of sperm treated in post meiotic tages from matings during the first 3 weeks and stages around meiosis from

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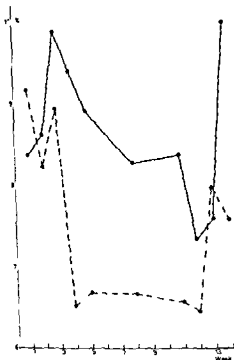


Fig 1 Percentages of intrauterine deaths in offsprings from matings at various periods after intraperitoneal injection of ^{90}Sr (—) and in controls (— — —)

combined to provide the material necessary for comparison between various stages of spermatogenesis

The combined data for the intraperitoneal series and controls are presented in Table 2 The males in one experiment (cf Table 3, series b) were not allowed any rest for 6 to 10 weeks but were mated throughout 13 weeks

The data in Table 2 are presented graphically in Fig 1 in which those from weeks 6 to 10 due to the paucity of the material are lumped together as a point for the 8th week The discrepancy as regards the curves in Fig 1 is difficult to explain as it appears to be due to a decrease in the control rather than to an increase in the ^{90}Sr series. This unexpected change in the control will be dealt with later

II Effect of intravenous versus intraperitoneal injection of ^{90}Sr The experiments of HENRICSON et coll (1962) indicate that the amount of ^{90}Sr in the testes is higher during the first hour when the injection is administered intraperitoneally than when given intravenously no difference was observed from the fourth hour It was therefore considered desirable to examine any possible

Table 2

Intrauterine death frequencies among offspring to males given intraperitoneal injections of ^{90}Sr and in controls (saline injections) related to matings at various weeks after injection

Week	Control group			Offspring to males given intraperitoneal ^{90}Sr		
	Implants	Dead	% dead	Implants	Dead	% dead
1	1 785	164	9 19	2 212	185	8 36
2	2 009	165	8 21	2 266	195	8 61
3	2 226	199	8 94	2 431	240	9 87
4	2 172	146	6 72	2 373	226	9 52
5	1 675	115	6 87	1 824	162	8 88
6	414	30	7 25	525	41	7 81
7	343	21	6 12	474	40	8 44
8	389	31	7 97	526	36	6 84
9	367	19	5 18	510	52	10 20
10	382	25	6 54	496	42	8 47
11	1 698	111	6 54	1 757	147	8 37
12	1 693	109	5 43	1 805	132	7 31
13	1 685	132	7 83	1 775	137	7 72
14	1 165	88	7 55	1 208	120	9 93
Total	18 003	1 355	7 53	20 182	1 755	8 70
1—3	6 020	528	8 77	6 909	620	8 97
4—5	3 347	261	6 78	4 197	388	9 24
6—10	1 895	126	6 65	2 531	211	8 34
11—14	6 241	440	6 05	6 545	536	9 19

matings during the 4th and 5th weeks, matings 10 to 15 weeks after injection gave sperm samples from cells in early spermatogonial stages at the time of injection

Considerable numbers of implants were needed for each week to detect variations between matings in consecutive weeks Since the preliminary test produced about 2 % excessive deaths in the ^{90}Sr series, as compared to the controls, about 2 000 implants per week per series were required These could not be obtained in one single experiment among other things due to the shortage of the supply of females In the course of the experiments the investigation was extended to include the effects of intravenous injections as well as the possible effects of the addition of Ca ions These experiments consisted of series with intraperitoneal injection of ^{90}Sr and control series as in the preliminary test In total four such sets of experiments were made, these inter se did not differ and hence could be



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Table 3

Intrauterine death frequencies among offsprings to males given ^{90}Sr intravenously compared with ^{90}Sr intraperitoneally and with parallel controls (saline injections)

Week	Control group			Intravenous injection group			Intraperitoneal injection group		
	Implants	Dead	% dead	Implants	Dead	% dead	Implants	Dead	% dead
Series (a)									
1—3	1 176	90	7 65	1 674	158	9 44	1 521	143	9 40
4	401	22	5 49	477	43	7 45	569	44	7 73
25—30	1 725	138	8 00	2 539	234	9 22	2 870	211	7 48
Series (b)									
1— 3	803	78	9 71	1 533	129	8 41	1 607	135	8 40
4— 5	800	63	7 87	1 103	104	9 42	1 060	96	9 06
6—13	2 999	194	6 47	3 954	313	7 92	4 101	332	8 10

Table 4

Intrauterine death frequencies among offsprings to males given intraperitoneal injections of ^{90}Sr ^{90}Sr with inactive Ca ions added and controls

	Control group			^{90}Sr injection group			^{90}Sr + Ca injection group		
Week	Implants	Dead	% dead	Implants	Dead	% dead	Implants	Dead	% dead
1— 3	1 750	160	9 14	1 720	108	9 19	1 659	153	9 22
4— 5	1 212	84	6 93	1 108	111	10 02	1 054	124	11 76
11—14	2 115	126	5 66	2 517	169	8 38	1 983	173	8 72

differences in the genetic effects of intraperitoneal and intravenous injections of ^{90}Sr This investigation comprised a total 80 male mice, thirty of which were given ^{90}Sr intraperitoneally and thirty the same amount intravenously while twenty mice that received saline intravenously served as controls

It was also considered worth while to investigate whether or not long term effects were involved after injection of the males In this experiment the males were held without females during the 5th to 25th weeks after injection and were then again mated to three females per week for 5 weeks Another experiment was focussed on the offspring during a long period of consecutive matings and for this purpose the males were mated to three females per week through 13 weeks

The data from these experiments are presented in Table 3 No clear differences between the groups given intravenous or intraperitoneal injection appeared to

Table 5

Intrauterine death frequencies among offspring to groups of males given intraperitoneal injections of (a) 10 μCi of ^{45}Ca of low specific activity (b) 20 μCi of ^{45}Ca of high specific activity and (c) 20 μCi of ^{45}Ca of high specific activity and with inactive Ca ions added

Week	Control group			Ca group			^{45}Ca + Ca group		
	Implants	Dead	% dead	Implants	Dead	% dead	Implants	Dead	% dead
Series (a)									
1—3	1 741	148	8 50	737	71	9 63			
4—5	1 059	78	7 37	414	71	9 94			
Series (b)									
1—3	1 597	122	7 64	1 624	125	7 70			
4—5	1 092	74	6 78	960	92	9 58			
Series (c)									
1—3				1 793	113	6 30	1 019	83	8 15
4—5				1 358	101	7 44	840	67	7 98

exist The number of fetuses tested a long time after injection proved to be too small to allow detection of any differences The total rates of intrauterine death in both ^{90}Sr series were significantly higher than in the control series

III Effect of addition of inactive Ca to ^{90}Sr injections The results of experiments that will be presented in next section suggested a reason for investigating whether or not the addition of inactive Ca ions to the ^{90}Sr would affect the rate of intrauterine death This experiment comprised two groups of males given ^{90}Sr and a control group Inactive Ca ions were added to one of the former groups (cf Table 1) The results are presented in Table 4 There were no obvious differences between the two ^{90}Sr series but they showed a total rate of intrauterine death significantly higher than the control group

IV Effect of ^{45}Ca As we had observed an increased rate of death in offspring to males given ^{90}Sr injections it was considered of interest to find out if ^{45}Ca would have a similar effect We had at first ^{45}Ca of low specific activity available The maximal amount at which the majority of males survived was 10 μCi during the first week they had a low fertility but from the second week it rose to a nearly normal value These males were used in matings for only 5 weeks A second experiment with ^{45}Ca of high specific activity was performed with 20 μCi intraperitoneally to each male

Table 6

Deaths occurring between birth and weaning among offspring to male mice given ^{90}Sr thirteen weeks before birth

Series	Born		Weaned		Lost		Number of litters
	♀	♂	♀	♂	♀	♂	
NaCl	472	465	416	405	56	60	140
^{90}Sr intraperitoneally	592	595	530	548	62	52	179
^{90}Sr intravenously	266	295	231	244	36	51	72

As the results indicated a higher effect with ^{45}Ca of low specific activity, we made a test with 20 μCi of ^{45}Ca of high specific activity and for half the number of males added inactive Ca ions In this experiment a parallel control was excluded due to lack of space This mistake made the results more or less useless as they indicated an overall lower rate of intrauterine death than in most other experiments

The data from these three tests with injections of ^{45}Ca (termed series a, b, c) are presented in Table 5 The material was divided in the same way as in previous tables of ^{90}Sr tests with data from the first 3 weeks and from the 4th to 5th weeks The data from series (a) and (b) obviously point in the same direction as for ^{90}Sr, with no detectable difference between controls and the ^{45}Ca group during the first 3 weeks, but an excessive rate of death in the ^{45}Ca series in the 4th to 5th weeks as compared to the parallel control (4th—5th week series a+b, $\chi = 8\ 4$, df $= 1$) The effect of ^{45}Ca thus seems to be similar to that of ^{90}Sr There are no significant indications of enhancement due to the presence of inactive Ca ions, but at the time of the experiment the difference appeared large enough to warrant analysis in relation to the effects of ^{90}Sr, as was described in section III

V *Test of survival from birth to weaning* Females mated the 10th week after injection were allowed to give birth to their litters in order to provide offspring for testing the possibly dominant effects appearing in later generations This constituted a small material but large enough to permit a study of survival between birth and weaning

The material was first analyzed by dividing the females into two classes those that lost the whole litter (mostly within the first two days after birth) and those from which at least one mouse was weaned The females in this latter group

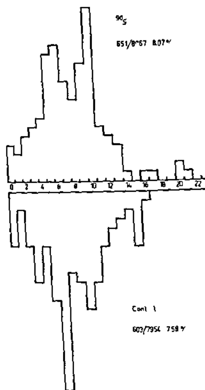


Fig. 2. Distribution of percentages (abscissa) of intrauterine deaths from F_2 males (ordinate: each cube in the columns representing one animal). The diagram include 127 F_2 males from the ^{90}Sr series and 108 males from the control series.

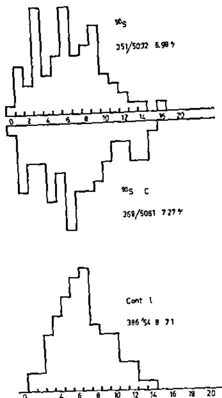


Fig. 3. Distribution of percentages (abscissa) of intrauterine deaths from F_2 males (ordinate: each cube in the columns representing one animal). The diagrams include 75 F_2 males from the ^{90}Sr series, 77 F_2 males from the ^{90}Sr + Ca series and 80 males from the control series.

proved their ability to take care of their young. Loss of some young in those litters may have been due to inferiority of the young, while the litters completely lost may have resulted from maternal incompetence (the rate of complete loss was about the same in the controls and the ^{90}Sr series). Such secondary influences had to be excluded and for that reason only data from females that had at least one young weaned were included (Table 6).

The rate of death before weaning is given separately for females and males. As may be seen from Table 6, the offspring to males that received intravenous and intraperitoneal injection of ^{90}Sr had a loss of 11.5 % while the control loss amounted to 12.4 %. It would therefore appear that the present data



Fig. 4. Distributions of percent ages (abscissa) of intrauterine deaths from F_3 males offsprings from the two F_2 males (B24 in upper diagram and B50 in lower diagram) which according to fig. 2 had produced high rates of deaths.

indicate no excessive rate of postnatal death among offspring to males receiving ^{90}*Sr injections.*

VI. Genetic effects in offspring to F_1 or F_2 males. An excessive rate of intrauterine death occurred in some of the preliminary experiments (Lüning et coll. 1963) in offspring to F_1 males whose father had been given ^{90}Sr injections, as compared to F_1 males from the control series. This result differed from what had been found in similar tests after roentgen irradiation. It was therefore of interest to confirm or invalidate the preliminary observations.

From two sets of experiments the F_1 offspring from the 10th week mating were allowed to produce an F_2 generation. Males among these were taken for tests of intrauterine death rate in matings to females from the CBA strain. Each male was mated to three females per week for 4 weeks. The F_2 males were coded so that their origin could be traced. The results are presented in Figs. 2 and 3. There are evidently no indications of any overall excessive rate of death in offspring to males from the ^{90}Sr or ^{90}Sr + Ca series.

Included in Fig. 2 are however three males from the ^{90}Sr series with a death rate around 20 %. One of these had produced only 15 implants, while the other two had 40 and 70, respectively. These last two males were brothers with code numbers B24 and B50. It seemed probable that they carried a mutation that led to a considerable rate of intrauterine death. The F_2 males mentioned were bred by CBA females to produce F_3 sons for further tests. When the F_3 males reached an age of about 60 days they were allowed a 4 week test period while mated to three females per week. Twenty eight sons to B24 and thirteen sons to B50 were tested, the results are presented in Fig. 4. None of the forty one males reached a level corresponding to their fathers (cf. Fig. 3). Any indication of dominant mutations could thus not be confirmed in these tests.

To determine whether there could have been some selection against F_1 offspring carrying mutations that would be manifested as intrauterine death we

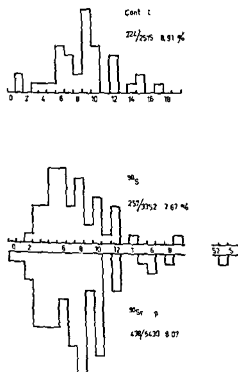


Fig 5 Distribution of percentages of intrauterine deaths from F_1 males as in figs 2 and 3 The F_1 males came from P (the treated fathers) males given ^{90}Sr intravenously and respectively intraperitoneally and from controls given saline

also made a test with F_1 males derived from the 10th week mating in the ^{90}Sr series given intravenous or intraperitoneal injection The F_1 males were tested in the same way as the F males with three females per week for 4 weeks (Fig 5) The total rate of intrauterine death was slightly higher in the controls than in the two ^{90}Sr series in spite of one semisterile male in the ^{90}Sr intraperitoneal series The semisterile male was further analyzed by matings to females of the CBA strain for production of offspring Male offspring were at an age of about 60 days tested in matings to three females per week for 4 weeks for analysis of intrauterine death Five of ten males had a normal death rate among their off pring while four males were sterile and one male made one out of twelve females pregnant out of eight implants this female had three dead

Female offspring to the semisterile male were used in breeding tests Only two out of nine females bred normally producing together a total of 100 live young eighty seven of which survived to weaning The remaining seven females produced 51 live young with none surviving to weaning

The detection of one semisterile male may not be taken as an indication of the effects of ^{90}Sr, as LÜNING & SHERIDAN (1964), using the same strain, observed a semisterile male from a control series. Neither did the test of F_1 males support the preliminary observation of an excessive rate of intrauterine deaths in offspring to F_1 males whose father had been given injection of ^{90}Sr.

Discussion

The results presented were obtained in experiments performed some years ago. The delay in reporting has been due to the results obtained in the control series and these will therefore be discussed first.

Several series of tests of the effects of ^{90}Sr and ^{45}Ca were made and in all but one series with controls. An examination of these control series, which were given saline injections, indicated considerable variations in the rate of intrauterine death. In matings the first 3 weeks after injection, the intrauterine death rate was higher than was normally observed in other control series. A drastic drop occurred from the 4th week, and the rate remained at a low level over a long period of time. The death rate was lower than in other experiments.

It has not been possible to discover the cause of these variations in the control groups. It might have been due to the intense mating scheme for the males. Other experiments in which the males had not been given saline injections failed to produce similar effects in early and late mating periods. It would thus appear that saline injections per se might have had some influence. In series performed during the past two years, the effect of saline injections do not appear to have produced similar results. No change in the rate of intrauterine death between the first 3 weeks of matings and the 4th to 5th weeks occurred in these experiments. The frequencies throughout were slightly higher than previously observed.

It may be mentioned that the dealer who supplied pellets during this period was unable to maintain the quality desired. Among the effects observed during the time of poor food supply was an increased rate of intrauterine death as well as decreased litter size.

This information has been presented at some length because *it is pertinent to* the analysis of the effects of ^{90}Sr. As mentioned earlier, the isotopes were given to the animals in saline solution. Both the ^{90}Sr treated mice and the controls were thus injected with saline solution of the same origin, but it seems to have had an effect only on the latter groups.

Contrary to the preliminary results (LÜNING et coll. 1963), the present investigation has failed to confirm any significant effects of ^{90}Sr in matings from the first 3 weeks *after injection*. The cell stages that could be affected during these three weeks were post meiotic, i.e. spermatids and spermatozoa.

Roentgen experiments have indicated (BATEMAN 1958) that the spermatid

stage is the most sensitive one. The peak value in the ^{90}Sr series occurred in the 3rd week but was not significantly higher than in the controls (Fig. 1).

HENRICSON et coll. (1962, 1964) investigated histologic and cytologic effects in testes and in fetuses obtained in matings at various times after the injection (peri- and post-meiotic stages at injection) in a series of experiments with the same CBA strain and ^{90}Sr from the same source as in the present experiments. These authors observed a decreased weight of the testes and that ^{90}Sr had stronger effects upon A type spermatogonia than on the B type. In testing fetuses they observed a greater variation in the number of chromosomes in the ^{90}Sr series than in the controls. These experiments are thus in agreement with the present data of excessive death among fetuses from matings in which the cells used were in peri- or pre-meiotic stages at the time of injection (matings 4th week and onwards).

The present investigation also included a small material in which the survival from birth to weaning of litters sired the 10th week after injection was being followed. We failed to observe any excessive deaths during the first 3 weeks after birth in offspring from the ^{90}Sr series.

An excessive number of deaths among offspring to F_1 males in the ^{90}Sr series had been noted in the preliminary tests (LUNING et coll. 1963). This result differed from previous experience in tests with roentgen irradiation reported in 1964 by LUNING & SHERIDAN. We made a series of tests in which offspring to F_1 or F_2 males were analyzed; this material was much more extensive than the preliminary one and has not confirmed its result. In one instance there were two F_2 brothers who both produced high rates of intrauterine death in their test matings. Careful analysis of F_3 sons of these two F_2 males gave no indication of the existence of a dominant factor causing excessive deaths. It must therefore be concluded that ^{90}Sr has not manifested dominant effects detectable in later generations. This conclusion is in agreement with findings from irradiations with roentgen and neutrons (unpublished results).

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SUMMARY

Inbred male CBA mice were injected with ^{90}Sr or ^{45}Ca and then mated, each male with three untreated females per week during five consecutive weeks. Most of the males were later used for another mating period during the 10th to 15th week post injection. More than 80 000 fetuses in the ^{90}Sr and ^{45}Ca series and about 58 500 in the controls were analyzed. The findings are considered in detail and their significance is discussed.

The detection of one semisterile male may not be taken as an indication of the effects of ^{90}Sr, as LÜNING & SHERIDAN (1964), using the same strain, observed a semisterile male from a control series Neither did the test of F_1 males support the preliminary observation of an excessive rate of intrauterine deaths in offspring to F_1 males whose father had been given injection of ^{90}Sr

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RADIOPATHOLOGY OF AMERICIUM 241

II Uptake in the developing teeth of rats

by

LARS HAMMARSTROM and AGNAR NILSSON

A previous study of the distribution of ^{241}Am in adult mice indicated that the dental pulp accumulated considerable amounts of this radionuclide (HAMMARSTROM & NILSSON 1970) Investigation on the distribution in the teeth of other actinide radioelements such as ^{239}Pu and [illegible]Th (JEE & ARNOLD 1960 ULLBERG et coll 1962) have revealed that these elements are deposited in the newly formed dentinal surface of the pulp chambers although no uptake in the soft tissues of the dental pulp has been reported It was therefore considered of value to study more in detail the uptake of ^{241}Am in the teeth The distribution of ^{241}Am in the developing enamel was also investigated So far there seems to be no detailed report on the distribution of any actinide element in the developing enamel

Material and Methods

Four 10 day-old rats of the Sprague Dawley strain were injected intraperitoneally with 0 26 ml of ^{241}Am citrate solution to give an individual dose of

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ZUSAMMENFASSUNG

Inzuchtige mannliche CBA Mause wurden mit ^{90}Sr *oder mit* ^{45}Ca *injiziert und dann* gepaart jede mannliche Maus mit drei unbehandelten weiblichen Mausen pro Woche wahrend funf aufeinander folgenden Wochen Die meisten mannlichen Mause wurden dann wieder zwischen der zehnten und funfzehnten Woche nach der Injektion gepaart Mehr als 89 000 Foten in den ^{90}Sr und ^{45}Ca Serien wurden untersucht und mit ungefähr 58 500 Kontrollfoten verglichen Die Befunde und deren Bedeutung werden eingehend erortert

RÉSUMÉ

Des souris males CBA de race pure ont reçu une injection de ^{90}Sr ou de ^{45}Ca puis chaque male a été accouple avec trois femelles non traitees par semaine pendant cinq semaines consecutives La plupart de ces males ont de nouveau ete accouples de la dixieme à la quinzième semaine apres l injection Les auteurs ont etudie plus de 89 000 foetus dans les séries traitées *par* ^{90}Sr *et* ^{45}*Ca et environ* 58 500 *dans la serie temoin Ils examinent en* detail les resultats et étudient leur signification

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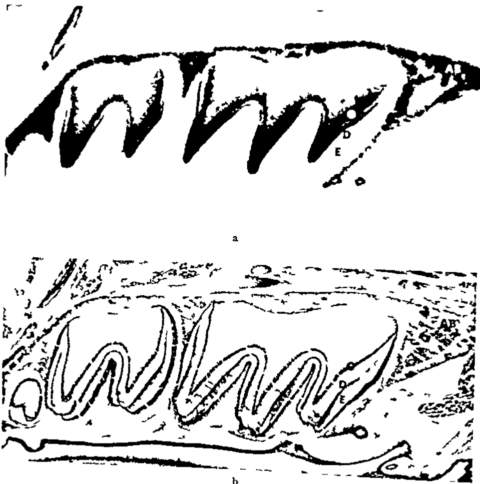


Fig 2 a) Detail of the whole body autoradiogram of the same rat as in fig 1 b) Corresponding section stained with hematoxylin and eosin Marked uptake in odontoblasts (O) and pulp surfaces of the dentine (D) low concentration at the surface of the enamel (E) the concentration in the odontoblasts and dentine seems to equal that of the alveolar bone (AB)

Results

The absorption of the intraperitoneally injected ^{241}Am was slow Radioactivity at 1 hour after injection could be registered only in the peritoneal cavity and the liver After 4 hours the concentration in the blood was high and thus the injected americium was distributed throughout the whole organism although there was no uptake in the skeletal tissues After 1 and 4 days however it lay in the

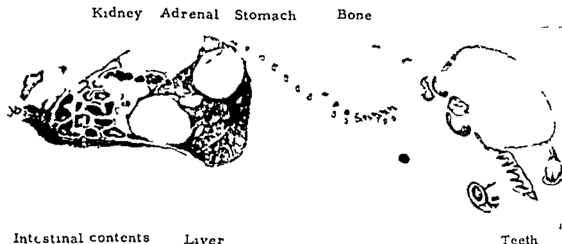


Fig 1 Whole body autoradiography Distribution of ^{241}Am in an 11 day old rat 24 hours after intraperitoneal injection Black areas indicate high concentration of radioactivity Deposition in bone and teeth high concentration in the liver and the intestinal contents low concentration in the kidney and hardly any radioactivity in the adrenal cortex

about 1 μCi One rat was killed at respectively 1 hour, 4, 24, and 96 hours after injection by immersion in a mixture of solid carbon dioxide and hexane (— 75° C)

Whole body autoradiography Sagittal (20 μ thick) sections through the whole frozen animals were cut and dried in a freezebox (— 10° C) The autoradiographic exposures were made by apposition against a roentgen film (Structurix, Gevaert) The exposure time was about 4 weeks for sections of animals of the different survival periods After exposure the sections were separated from the roentgen films and stained with hematoxylin and eosin (ULLBERG 1954, 1958) The films were developed, fixed and rinsed

Microradiography Twelve (10 μ thick) sections of the head of the rats with survival periods of 1 day and 4 days were cut in a freezebox (— 10° C) and placed on Scotch tape with a polyvinyl chloride backing (No 688, Minnesota Mining and Manufacturing Co) The sections were dried in the freezebox (— 10° C) and dry mounted on glycerine treated nuclear emulsion plates (emulsion thickness 10 μ, Ilford G 5) After exposure for 4 weeks, the Scotch tape was dissolved in xylene, the nuclear emulsion plates developed, fixed and rinsed, and finally the sections still remaining on the emulsion surface were stained with hematoxylin and eosin The method has been described in detail by HAMMARSTROM et coll (1965)



Fig 4 Detail of section shown in fig 3 indicating superficial uptake of ^{241}Am in the enamel (E) The concentration is low under the tall ameloblasts (TA) but increases under the short ameloblasts (SA) towards the tip of the cusp the accumulation in the odontoblasts (O) seems to be divided into proximal distal and predentinal (PD) parts

hours after its administration only faint autoradiographic traces at the surface of the enamel and in the ameloblasts were evident (Fig 3) The concentration at these sites was markedly lower where matrix formation occurred than in the areas where the enamel had attained its final thickness (Figs 2 and 4)

Four days after injection of americium some radioactivity was seen in a wide zone in the enamel about half way to the dentine enamel junction (Fig 6) It should however be kept in mind that at all the intervals studied the concentration in the enamel was low in comparison with that in the developing dentine and odontoblasts.

Discussion

The present investigation has confirmed the previous observations (Hammarstrom & Nilsson 1970) of a marked accumulation of ^{241}Am in the cells of the dental pulp None of the other actinide radioelements studied have been reported



Fig 3 Microautoradiogram Distribution of ^{241}Am in the first upper molar of an 11 day old rat 24 hours after intraperitoneal injection Uptake in the odontoblasts (O) predentine (PD) and alveolar bone (AB) some spots with high concentration are spread in the pulp (P) there is hardly any uptake at the surface of the enamel (E)

dental tissues and in the periosteal and endosteal surfaces of the bone The distribution in the body was similar to that in adult mice after intravenous injection, which has recently been described (HAMMARSTROM & NILSSON 1970) It was noted that ^{241}Am did not accumulate in the adrenal cortex of young rats, as it did in adult mice (Fig 1)

In the developing molar teeth, the americium was accumulated in the dental pulp mainly in the odontoblasts, and these cells displayed about the same concentration as that of the surface of bone (Fig 1) The americium in the odontoblasts was partly located in the distal part of the cells close to the dentine and partly on the pulpal side of the nuclei However, this latter uptake might also have been located in the pulpal tissue close to the odontoblasts and not within those cells There was in addition marked accumulation in the predentine 24 hours after injection (Fig 2) Three days later this zone of radioactivity appeared in the mineralized dentine The americium seemed to remain unchanged in the odontoblasts during the four days of investigation

Little of the ^{241}Am was taken up in the developing enamel Twenty four



Fig 6 Microautoradiogram 4 days after injection The americium is located in the enamel (E) about half way to the amelodentine junction (AD) The concentrations in the odontoblasts (O) and in the dentine (D) remain high

Americium is selectively concentrated at the resting and resorbing surfaces of bone (TAYLOR et coll 1961 HERRING et coll 1962) and good conformity has been noted between areas with a marked concentration of americium and those with a strong PAS positive reaction (HERRING et coll 1962) It is therefore interesting to note that the concentration of americium in the enamel increased at the stage of maturation during which organic material and water is removed and minerals are deposited (DEAKINS 1942 WEINMANN et coll 1942 and others) The surface area where americium appeared 24 hours after injection has proved to be PAS positive (SUGA & GUSTAFSON 1963)

The mechanism of binding of americium to the skeletal tissues is still obscure The fact that there was a low concentration of ^{241}Am in the developing enamel is a good illustration implying that the mineral component is of little or no importance in the binding mechanism Recent investigations have indicated that it is bound in bone to some glycoproteins (HERRING et coll 1962 CHIPPERFIELD & TAYLOR 1968) Whether this holds also for its binding to the dental tissues remains to be settled The pattern of deposition of ^{241}Am in the enamel indicated



Fig. 5. Microautoradiogram. Distribution of ^{241}Am in the first (M1) and second molar teeth (M2) of a 14 day old rat 4 days after intraperitoneal injection. High concentration of radio activity in the pulpal surface of the dentine (D), in the odontoblasts (O) and the interdental bone septum (BS).

to have accumulated in the odontoblasts (JEE & ARNOLD 1960). It is still unsettled whether this accumulation is specific for americium or whether the methods used for the localization of other actinides have failed to detect it in these cells.

The autoradiographic techniques used in the present investigation were such that tissue was never in contact with any liquid medium before autoradiographic exposure, so that redistribution or isotope loss before recording in the photographic emulsion was prevented. The marked accumulation of americium in the odontoblasts may indicate that americium is taken up in the osteoblasts as well, since these cells are metabolically and functionally similar. No such uptake appears, however, to have been reported.

It might be expected that the high energy alfa radiation of americium would induce neoplastic growth of the odontoblasts and that special attention should be given to the late biologic effect of ^{241}Am on the dental pulp. It should, however, be remembered that the mature odontoblasts are highly radioresistent (KAININS 1954). Injection of ^{239}Pu in dogs has previously been shown to stimulate secondary dentine formation but no tumours developed (JEE & ARNOLD 1960).

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no definite relation to protein matrix deposition or mineral deposition as indicated by autoradiography of labelled amino acids and radiocalcium (BELANGER 1957, GREULICH & SLAVKIN 1965, HAMMARSTROM & LINGE, to be published)

The latency in the accumulation of ^{241}Am at the surfaces of bone and in odontoblasts and ameloblasts may suggest that it be incorporated into a larger molecule with an affinity for these tissues Few substances have a marked affinity for the postsecretory ameloblasts, the metabolism of which is very little known The only metal so far known to accumulate in the ameloblasts of rat molar teeth during maturation is iron (HAMMARSTROM & JONSSON, in preparation) It is therefore interesting that plutonium, and probably americium as well, are transported in the blood bound to transferrin (BOOCOCK & POPPLEWELL 1965, STOVER et coll 1968) This may indicate that the accumulation in the ameloblasts occurs in the form of an americium transferrin complex

SUMMARY

Young rats were injected intravenously with ^{241}Am citrate solutions and investigated at different times by whole body autoradiography and microradiography The techniques are described and the findings discussed in detail

ZUSAMMENFASSUNG

Junge Ratten wurden mit ^{241}Am Citrat injiziert und in verschiedenen Zeitabstanden mittels Korperautoradiographie und mittels Mikroradiographie untersucht Die Methodik und die Resultate werden detailliert beschrieben

RÉSUMÉ

Les auteurs ont injecte a de jeunes rats par voie intraveineuse des solutions de citrate ^{241}Am et les ont examines a differentes dates par auto radiographie et microradiographie du corps entier Ils décrivent les techniques et etudient en detail les resultats

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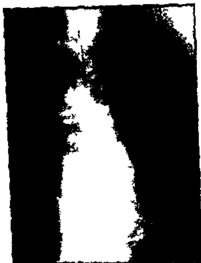


Fig 1 Roentgenogram for local za
i on n Case 2

each field position was printed out as a full size plan with a *coded contour plot* displaying the dose distribution

The mean tumour dose was normally prescribed on 95 to 98 per cent of the maximum so as to ensure an adequate dose to the whole of the tumour volume The plans considered here were for 45° wedged fields rotated through two arcs of 100 or 110

Method and Results A continuous row of LiF teflon microrod dosimeters of 1 mm diameter and 6 mm length were inserted into 1 mm bore nylon tubing

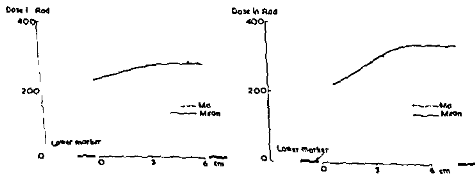


Fig 2 Variation in dose along the oesophagus

FROM THE DEPARTMENT OF RADIOTHERAPEUTICS, UNIVERSITY OF CAMBRIDGE AND RADIOTHERAPEUTIC CENTRE, ADDENBROOKE'S HOSPITAL, CAMBRIDGE, ENGLAND

DOSE MEASUREMENTS IN OESOPHAGUS IN COBALT 60 ROTATION THERAPY

by

A. C. McEwan and T. K. Wheeler

Radiation doses in bladder and oesophagus tumour sites have been measured with thermoluminescence dosimeters in a number of patients treated by rotation with cobalt 60 radiation and compared with the doses prescribed. While for the bladder cases agreement to within a few per cent was obtained (McEwan 1969), the deviation from the prescribed doses varied more widely for the oesophagus cases. Two representative oesophagus cases are considered in more detail.

Production of treatment plans. Rotation treatment plans were produced using the University of Cambridge Titan computer by the method described by MacDonald (1965), based on isodose curves normalized to 100 per cent at the centre of rotation, with corrections where necessary for oblique incidence and lung. The use of this multi access system for treatment planning has been described by Haybittle & Houston (1968). The lung correction was made by an isodose chart shift method using a shift of 0.33/cm. From depth dose and decrement data for selected field sizes held in store the computer selected the decrement data appropriate to an input field size by interpolation (cf. Orchard 1964), and from this data computed a dose matrix. The sum of the dose matrices for

Submitted for publication 17 October 1969.

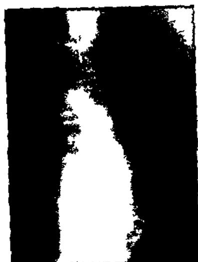


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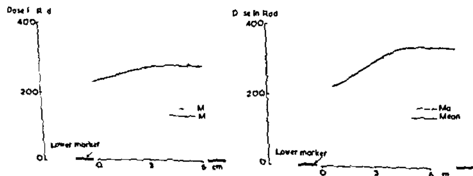


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low dose to the region of the dose maximum occurring over a distance of about 4 cm

The e measurements demonstrate that for fields where lung corrections are necessary reasonable agreement between prescribed and actual doses may be obtained for positions near the centre of the treatment field, but that further away from the field centre changes in the lung volume, particularly in the basal parts of the lung may give rise to significant errors Discrepancies may also arise where the treated organ is irregular in shape so that the treatment volume does not coincide with the tumour volume

Acknowledgements

We wish to thank Professor J S Mitchell and Dr J L Haybittle for their interest in this work A C M acknowledges the award of a New Zealand Government Research Fellowship

SUMMARY

A method is described for measuring the variation in tumour dosage which differs from a prescribed plan using thermoluminescent lithium fluoride microrod dosimeters Two representative cases are considered in detail

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben die Abweichung der Tumordosis von einem vorher bestimmten Plan mit Hilfe von Lithium Fluorid Mikrostabdosimetern zu bestimmen Zwei repräsentative Fälle werden im einzelnen betrachtet

RÉSUMÉ

Description d une methode mesurant la difference entre la dose à la tumeur et la dose prevue dans le plan de traitement utilisant des microdosimetres thermoluminescents au fluorure de lithium Deux cas servant d exemples sont étudies en détail

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Table

Summary of measurements in two cases

	Prescribed doses in rad		Number of dosimeters averaged	Measured doses in rad	
	Mean	Maximum		Mean	Approx. range
Case 1	250	272	11 (5)	259 (273)	235—270
Case 2	300	327	12 (6)	298 (335)	235—335

with short metal markers above and below the line of dosimeters so that their position could be seen in the roentgenograms (Fig. 1). The end of the nylon tubing was heat sealed and inserted in a Ryle's tube for measurements in the oesophagus. The standard deviation of a large number of dosimeters irradiated to the same dose was about 5 per cent.

Measurements in two cases are summarized in the Table. There was some change in dose along the line of the dosimeters (Fig. 2), particularly in Case 2, and the average doses recorded in the dose maximum areas are listed separately (bracketed). In both cases, the treatment fields extended beyond the markers.

Discussion

Since the lung corrections for the rotation fields were assessed on the patients at the level of the centre of the treated volume, and because of the increase in lung volume superiorly and decrease inferiorly in the region of the heart, the treatment plan to some extent under-corrected for the lung superiorly and over-corrected inferiorly. A change in the average lung path from 6 to 4 cm at this radiation energy would account for a change in dose of about 6 to 7 per cent, so that part of the observed fall off in dose inferiorly may be attributed to the decrease in lung path. Where the oesophagus deviated significantly from the medial axis or moved anteriorly away from the spine, the dosimeters may have been outside the region of the highest dose indicated in the treatment plan.

In Case 2, where the treatment field and dosimeters extended lower in the oesophagus than in Case 1, the deviation of the oesophagus from the midline was evident in the roentgenogram and this would partly account for the low doses recorded in this region. Since also in Case 2 the lower dosimeters were below the diaphragm, the doses in this region were further lowered by the magnitude of the treatment plan lung correction (about 20 per cent). The doses recorded by the lower dosimeters, about 70 per cent of the prescribed mean dose, are therefore seen to be not unreasonable for this position, with the transition from the

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STRAHLENBIOLOGIE Von E Kálman L Sztanyik E Unger and V Várterész Herausgegeben von V Várterész 603 Seiten, 121 Abbildungen und 22 Tabellen Akadémiai Kiadó Budapest 1966 Preis 17 Dollar

This book is a translation of a work published in Hungarian in 1963 It is an introduction to radiobiology intended mainly for doctors working in the fields of radiology and radiation protection A short review of radiation physics and dosimetry is followed by sections on the theory of primary processes in connection with radiation and the biochemistry of radiation effects Other sections deal with the pathophysiology of radiation and genetic problems the cancer and leukaemia inducing effects of radiation chemical radiation protection the toxicology of radioactive substances and the symptomatology of radiation effects in man and their treatment The majority of the publications up to 1960 or thereabouts are included in the bibliography and the book contains a good index As an introduction to the subject the presentation is sometimes too long and undifferentiated for non specialists

Bernhard Tribukait

RADIATION RESEARCH Proceedings of the Third International Congress of Radiation Research held at Cortina d Ampezzo Italy June—July 1966 Edited by G Silini 927 pages 388 figures and 67 tables North Holland Publishing Company Amsterdam 1967 Price 120 guilders

At the Third International Congress of Radiation Research particular attention was given to the radiation effects on the atomic molecular and cellular levels The book presents the lectures read by leading scientists at the twelve symposiums held at the Congress The following subjects were treated energy deposition at the atomic level radiation induced radicals radiation chemistry of water radiation effects on artificial polymers radiation effects on macromolecules of biological significance radiation sensitivity of genetic structures radiation mutagenesis mechanisms of cell recovery radiation effects on cell populations radiation and the origin of life biological effects of UV irradiation photodynamic effects These papers containing good reference lists provide an up to date survey of these fields

A comparison with the second international congress of radiobiology held four years earlier gives a good idea of the rapid developments that have taken place in the respective divisions of radiobiology The book can be warmly recommended to libraries and institutes of radio biology and radiophysics

Bernhard Tribukait

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